THE CEREBELLAR VERMIS ROBUSTLY MODULATES NEURAL ACTIVITY IN THE INFERIOR COLLICULUS

by

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Abstract of the Dissertation

To survive in an ever-changing world, animals need to rapidly detect and appropriately respond to external stimuli. In the auditory system, the inferior colliculus (IC) is well-positioned as an obligatory auditory hub involved with making acousticomotor responses to initiate these behavioral responses to auditory stimuli. However, it is not well understood how the relevant contextual information needed to detect and respond appropriately to stimuli is conveyed. In this thesis, I investigate the cerebellar vermis as one possible source of this contextual information.

My experiments revealed that optogenetic stimulation of the cerebellar vermis robustly modulates the majority of neurons throughout IC in awake mice head-fixed on a treadmill. Because I was to monitor the movement of the treadmill, I also found that vermis stimulation produces a similar behavioral response as auditory stimuli, and that vermis evoked activity in the IC is in part related to this motor response. Furthermore, my results show that animal running modulates IC activity by reducing auditory and vermis responses, while increasing spontaneous activity.

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This thesis is dedicated to my mom and dad, Drs. Qing Jiang and Hong Sima, and to my sister, Renee Sima.

The idea that there is generality
in the specific is of far-reaching importance.

- Douglas Hofstadter

No man ever steps in the same river twice,
for it’s not the same river and
he’s not the same man.

- Heraclitus

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Chapter 1. Introduction

In order to survive, animals need to detect stimuli in the external environment and respond with appropriate behaviors. For a mouse, a rustling sound in a bush might signal a lurking predator and, in a fast-changing world, the animal must both quickly identify the salient stimulus and react appropriately. Or for a graduate student, footsteps approaching from behind may mean either an adviser hungry for data or a labmate hungry to grab lunch – the student needs to detect and respond differently to either challenge. In order to initiate behavioral responses to acoustic stimuli rapidly, the auditory system would need to have the relevant contextual information and built-up predictions about the state of both the environment and the animal.

It has been proposed that neurons in the auditory midbrain, the inferior colliculus (IC), can act as a filter for auditory stimuli requiring immediate action, such as sounds associated with predators, prey, and conspecifics (Casseday & Covey, 1996). But how does the IC, in specific, and the brain, more generally, acquire the contextual information relevant for detecting and acting on salient events?

This thesis hopes to further our understanding of these issues. To this end, in Chapter 2 I investigate how the cerebellum, which integrates multisensory information and serves predictive functions, modulates IC activity. In Chapter 3, I then analyze how animal motor responses produced by auditory and vermis stimulation affect IC activity. In Chapter 4, I examine how animal locomotion modulates IC activity. Below I will summarize the scientific context that led to the studies presented in Chapters 2 – 4.

The inferior colliculus is an auditory hub that integrates multisensory and contextual information

The inferior colliculus (IC) is well-positioned for generating appropriate behavioral responses to external stimuli. The IC is a nigh obligatory auditory hub in the midbrain for ascending acoustic information arising and converging from the cochlear nucleus, superior olivary complex, and the nuclei of the lateral lemniscus (Beyerl, 1978; Faye-Lund & Osen, 1985; Winer & Schreiner, 2005). This ascending input primarily projects to the central nucleus of the IC (CIC), which relays auditory information to the ventral region of the medial geniculate body of the thalamus (Faye-Lund & Osen, 1985; Winer & Schreiner, 2005). The IC shell region, comprised of the external cortex of the IC (ECIC) and the dorsal cortex of the IC (DCIC), receives top-down feedback from the auditory cortices (Games & Winer, 1988; Huffman & Henson, 1990; Schofield, 2009). In addition, the IC receives cholinergic, noradrenergic, serotonergic, and dopaminergic inputs, among its neuromodulatory influences (Ayala & Malmierca, 2015; Klepper & Herbert, 1991; Motts & Schofield, 2010, 2011; Peruzzi & Dut, 2004; Schofield & Motts, 2009).

The IC receives significant non-auditory signals as well. Inputs from somatosensory cortex and the dorsal column nuclei are found almost exclusively within neurochemically distinct modules distributed throughout the rostrocaudal extent of the ECIC (Lesicko & Llano, 2019; Lesicko, Hristova, Maigler, & Llano, 2016; Stebbings, Lesicko, & Llano, 2014). Specifically, neurons in these modules stained positive for glutamic acid decarboxylase-67 (GAD-67), acetylcholinesterase (AChE), parvalbumin (PV), nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d), and

cytochrome oxidase (CO) (Lesicko & Llano, 2019). Recordings in cat ECIC found responses to both auditory and somatosensory stimuli within the same single units (Aitkin, Kenyon, & Philpott, 1981; Aitkin & Zimmermann, 1978). In addition, IC activity is modulated depending on eye orientation (Groh, Trause, Underhill, Clark, & Inati, 2001), visual stimuli, saccadic eye movements (Bulkin & Groh, 2012; Porter, Metzger, & Groh, 2007), and impending reward (Metzger, Greene, Porter, & Groh, 2006). These other non-auditory signals are found throughout the IC, but are stronger in regions with fewer neurons tuned to specific frequencies – likely in the IC shell (Gruters & Groh, 2012a).

The inferior colliculus contributes to defensive behaviors and acousticomotor responses

In addition to playing a role in the auditory system, the IC also contributes to acousticomotor control and defensive behaviors (Huffman & Henson, 1990). Increases in electrical stimulation intensity of the IC produces a gradation of defensive behaviors, from alertness to freezing and finally to escape (Blanchard, Griebel, Pobbe, & Blanchard, 2011; Brandão, Melo, & Cardoso, 1993). Pharmacological manipulations disinhibiting the IC elicits defense reactions, like running (Bagri, Di Scala, & Sandner, 1992; Brandão, Tomaz, Leão Borges, Coimbra, & Bagri, 1988). Optogenetic stimulation of auditory cortex inputs in the IC shell region also induces animal running (Xiong et al., 2015).

The IC is required for prepulse inhibition of the acoustic startle reflex, where a weak, non-startling stimulus preceding a strong, startling sound suppresses the magnitude of the startle movement (Koch, 1999; Lauer, Behrens, & Klump, 2017). The role the IC

plays in behavioral modification of startle is thought to be mediated via the IC's projections to cholinergic neurons in the pedunculopontine tegmentum (PPTg), which are involved in arousal (Lauer et al., 2017). Caudal pontine reticular nucleus (PnC) neurons synapse onto spinal cord motoneurons (and their upstream interneurons) that produce the startle response, and IC activation of an inhibitory cholinergic input on PnC neurons may inhibit the startle (Diederich & Koch, 2005; Lauer et al., 2017; Yeomans, Lee, Yeomans, Steidl, & Li, 2006).

In addition, the IC has known connections to brain regions involved with orienting behavior. The ECIC projects to the superior colliculus, which receives auditory, tactile, and visual input, and plays a role in orienting the head, eyes, and pinna (Comoli et al., 2012; Huffman & Henson, 1990). In addition, the IC makes a pontocerebellar input to the cerebellar vermis, which is involved with saccadic eye movements, via the dorsolateral pontine nucleus (Aitkin & Boyd, 1978; Azizi, Burne, & Woodward, 1985; Huffman & Henson, 1990; Kheradmand & Zee, 2011; Moore & Goldberg, 1966).

The integrative and predictive cerebellum

The cerebellum is classically regarded as a structure that coordinates movements and is involved in motor learning. Cerebellar lesions in human patients can cause deficits in posture, gait ataxia, and oculomotor disorders (Stoodley & Schmahmann, 2010). However, converging lines of clinical, anatomical, imaging, and behavioral evidence are highlighting the cerebellum's role in non-motor functions (Bodranghien et al., 2015; Schmahmann, 2004; Schmahmann & Caplan, 2006).

The cerebellar cortex is comprised of circuit motifs with that includes parasagitally organized zones of inhibitory Purkinje cells (PCs) (Apps & Hawkes, 2009; Apps et al., 2018; Sarpong et al., 2018). Mossy fiber inputs from the brainstem excite granule cells, which in turn make excitatory contacts onto PCs. Each PC also receives a single climbing fiber input from the inferior olive, which provides an error signal if the sensory consequence of an action did not match what was predicted (Ito, 2006). Importantly, these error signals allow the cerebellum to refine internal models about the world and predict what will happen in the future based on past experience. PCs are the sole output neurons of the cerebellar cortex, and innervate neurons in the cerebellar nuclei. PCs are GABAergic and fire at high spontaneous rates, and thus tonically inhibit the activity of cerebellar nuclei neurons. Synchronized pauses in PC simple spike activity have been shown to strongly impact output of the cerebellar nuclei (Person & Raman, 2012; Ramirez & Stell, 2016) and are involved with cerebellar-dependent learning paradigms (Lee et al., 2015).

The cerebellar vermis, located medially in the cerebellar cortex, and its associated cerebellar output nucleus, the fastigial nucleus (FN), are particularly involved with limbic, cognitive, as well as motor behaviors (Adamaszek et al., 2016; Barton, 2012; Koziol et al., 2014; Schmahmann & Caplan, 2006; Strata, 2015; Zhang, Wang, & Zhu, 2016). The vermis is involved with whole-body posture and movement, and receives somatosensory inputs from the spinal cord (Coffman, Dum, & Strick, 2011; Manto, Gruol, Schmahmann, Koibuchi, & Rossi, 2013). In human functional imaging studies, vermis lobule VII is implicated in cerebellar-limbic circuitry (Stoodley & Schmahmann,

2009). Indeed, the cerebellar vermis was found to evoke responses in the hippocampus, septum, and amygdala when electrically stimulated (Snider & Maiti, 1976).

Functionally, the cerebellar vermis has known roles in the prediction of sensory consequences of movement, orienting, long-term modification of startle (Baumann et al., 2015a; Blakemore, Frith, & Wolpert, 2001; Bodranghien et al., 2015; Koziol et al., 2014; Leaton & Supple, 1986; Maschke, Drepper, Kindsvater, & Kolb, 2000; Steriade, 1995). Vermis stimulation is also known to produce arousal and cortical activation via the reticular activating system (Steriade, 1995). Thus, the cerebellar vermis is well-positioned to provide integrated, predictive, and contextual signals for generating appropriate behaviors to relevant stimuli.

Known interactions between the cerebellar vermis and the auditory system

In a seminal study conducted at Johns Hopkins University and published in 1944, Ray Snider and Averill Stowell discovered that neurons in the cerebellar vermis responded to teleceptive stimuli, including sound (Snider & Stowell, 1944). This "auditory receiving area" was specifically located in the midvermis, lobules VI/VII, and also receives visual and tactile input (Snider & Stowell, 1944). In the decades that followed, a number of different groups recorded responses of granule cells and Purkinje cells to different auditory stimuli (Aitkin & Rawson, 1983; Altman, Bechterev, Radionova, Shmigidina, & Syka, 1976; Deura & Snider, 1964; Freeman, 1970; Huang & Liu, 1990; Jastreboff & Tarnecki, 1975; Lorenzo, Velluti, Crispino, & Velluti, 1977; Mortimer, 1975; Radionova & Shmigidina, 2015; Shofer & Nahvi, 1969; Wolfe, 1972). Units with auditory responses in the vermis have broad tuning curves, are facilitated by

binaural sound presentation, and tend to prefer sound sources directly in front of the animal (Aitkin & Boyd, 1975; Aitkin & Rawson, 1983; Altman et al., 1976). These auditory response properties suggest that cerebellar output of these neurons may modify orienting movements towards the sound source (Huffman & Henson, 1990).

Interestingly, the auditory inputs to the midvermis originate from the IC. The ECIC in particular projects to the dorsolateral pontine nucleus (DLPN), which in turn sends mossy fibers to the midvermis lobules VI/VII (Azizi et al., 1985; Azizi, Mihailoff, Burne, & Woodward, 1981; Huffman & Henson, 1990; Kawamura & Hashikawa, 1981). The auditory cortex also sends projections to the same region of the DLPN, but contacts neurons involved in sending a separate mossy fiber input to the paraflocculus (Azizi et al., 1985). Electrical stimulation of the IC, but not the auditory cortex, evokes short-latency responses in the midvermis (Azizi et al., 1985). In addition, in their original study, Snider and Stowell found that bilateral lesioning of the inferior colliculus abolishes the response to auditory clicks in the cerebellar vermis (Snider & Stowell, 1944). Thus, the cerebellar vermis receives auditory input from the IC and is poised to modify its activity in turn.

A few previous studies investigated the effect cerebellar stimulation has on the auditory system. Electrical stimulation, particularly of vermis lobules V-VII, was found to reduce all components of the auditory brainstem response (ABR) (Crispino & Bullock, 1984). Cerebellar electrical stimulation was also reported to diminish cochlear microphonics and auditory nerve action potentials, while cooling the cerebellar cortex increased their respective amplitudes (Velluti & Crispino, 1979).

The cerebellar vermis is also implicated in auditory dysfunction. In human imaging studies, tinnitus was associated with vermis activity (Lanting, de Kleine, Eppinga, & van Dijk, 2010; Lanting, De Kleine, Langers, & Van Dijk, 2014). Tinnitus (sound perception in the absence of an external physical source) and hyperacusis (increased sensitivity to normal sound levels) involves an enhanced auditory network composed of the IC, cerebellum, and limbic system (Chen et al., 2015) as well as increased functional connectivity of vermis lobule VII to the cerebral cortex (Feng et al., 2018). Of interest is the fact that IC becomes hyperactive following hearing loss-related tinnitus, and exhibits increased spontaneous firing rates and evoked responses to sounds (Xiong et al., 2017). While gain amplification of the central auditory system is hypothesized to compensate for reduced signaling from the cochlear in hearing loss, the source of the gain change is unknown (Auerbach, Rodrigues, & Salvi, 2014; Sedley, 2019).

Given the substantive, if understudied, connection between the cerebellar midvermis (lobules VI/VII) and the auditory system, I tested the functional connectivity of the cerebellar vermis with the IC. In Chapter 2, I describe the effects of optogenetic stimulation of the midvermis on extracellular activity throughout the IC in awake, head-fixed mice.

The acoustic startle response and other responses to salient stimuli

Both the cerebellar vermis and IC are involved with modification of the startle behavior, which is a rapid contraction of facial and body musculature in response to

strong, unexpected stimuli (Koch, 1999; Lauer et al., 2017; Leaton & Supple, 1986; Maschke et al., 2000). The acoustic startle response (ASR) is typically elicited by loud, sudden sounds, and similar behavioral responses can also be produced by strong, unexpected tactile or vestibuli stimuli (Yeomans, Li, Scott, & Frankland, 2002).

Although its functional significance is debated, the startle response probably serves to protect the animal from physical harm, particularly blows to the head, and is an example of a rapid behavioral response to salient stimuli (Yeomans et al., 2002). When humans startle, they typically close their eyes, hunch their shoulders and back, and retract their limbs, while tensing their muscles (Yeomans et al., 2002). Similarly, rats retract their head towards their body and arch their back (Yeomans et al., 2002). In both species, a startle movement reduces the size of the body, particularly limiting exposure to vulnerable areas like the neck and abdomen, while stiffening muscles to resist possible blows. It is also possible that the startle response serves to interrupt ongoing behaviors and allow the animal to prepare for action (Koch, 1999). However, there are potential costs associated with startling in the form of short-term decreased motor coordination, attention, and visual input as a result of whole-body muscle contraction and eye closure.

The startle response can be modified adaptively depending on the state of the animal and context of stimulus presentation (Koch, 1999; Lauer et al., 2017). If a loud noise is preceded by a quieter sound, the startle is reliably inhibited; this prepulse inhibition (PPI) depends upon the IC (Fendt, Li, & Yeomans, 2001; Koch & Schnitzler, 1997; Lauer et al., 2017; Yeomans et al., 2006). Animals can also habituate to startling stimuli in the long-term, a process that requires the cerebellar vermis (Leaton & Supple, 1986; Maschke et al., 2000).

It is worth noting that startle responses are reflexive and involuntary. However, substartle, but salient, stimuli can also produce motor action coupled with cortical activation, suggesting that saliency detection entails a built-in reaction to the stimulus alongside its perception (Novembre et al., 2018).

In Chapter 3, I investigate the relationship of IC neural activity with the whole-body movements mice make in response to auditory clicks and vermis stimulation. The movements produced by both of these stimuli are similar, and consist of a fast component and a slow component that correspond to a startle and a preparatory movement, respectively.

Behavioral state modulation of sensory areas

Behavioral state changes the functional demands of the animal and thus the functioning of its nervous system. In the visual system, locomotion increases responsivity of primary visual cortex (V1) to visual stimuli, possibly to account for increased optic flow (Dadarlat & Stryker, 2017; McGinty, Lardeux, Taha, Kim, & Nicola, 2013; Niell & Stryker, 2010). The visual thalamus carries locomotion signals representing a combination of visual and running speed, while activity in the lateral posterior nucleus (the rodent homolog of the pulvinar) may signal the discrepancy between visual and self-motion (Roth et al., 2016).

While the auditory system is also modulated by behavioral state, its responses are suppressed instead of enhanced during locomotion. Responses to auditory stimuli and input from auditory thalamus are attenuated in auditory cortex while the animal is

running (Nelson & Mooney, 2016; Nelson et al., 2013; Schneider, Nelson, & Mooney, 2014; Zhou, Liang, et al., 2014). In the auditory thalamus, sound evoked sounds are also reduced (Williamson, Hancock, Shinn-Cunningham, & Polley, 2015). In addition, self-generated sounds are cancelled out in both the auditory cortex and the dorsal cochlear nucleus (Rummell, Klee, & Sigurdsson, 2016; Singla, Dempsey, Warren, Enikolopov, & Sawtell, 2017). However, despite studies showing behavioral state modulation of the auditory system in the cochlear nucleus, medial geniculate bodies, and auditory cortex, no studies have looked at the effect of locomotion on the IC. As such, in Chapter 4, I investigate whether and how spontaneous animal running during auditory clicks and vermis photostimulation affects IC activity.

Chapter 2: Cerebellar vermis photostimulation modulates inferior colliculus neural activity

Introduction

Animals need to detect signals in the external environment and to respond with appropriate behaviors depending on whether the stimulus is threatening, neutral, or rewarding. The inferior colliculus (IC) is an auditory system hub that is important for acousticomotor behaviors relevant for survival, including startle, escape, and orienting (Branda, Troncoso, Silva, & Huston, 2003; Brandão et al., 2005; Huffman & Henson, 1990; Xiong et al., 2015), and is well-positioned to help the animal detect and respond to external stimuli.

The IC integrates different contextual signals in addition to ascending auditory information from the periphery (Winer & Schreiner, 2005). The dorsal cochlear nucleus provides auditory inputs with information about self-generated sounds produced during animal movement (Singla et al., 2017; Wigderson, Nelken, & Yarom, 2016). Descending inputs from the auditory cortex to the shell region of IC can provide feedback (Games & Winer, 1988; Huffman & Henson, 1990; Schofield, 2009). IC receives different neuromodulatory inputs, including from cholinergic, noradrenergic, serotonergic, and dopaminergic sources (Ayala & Malmierca, 2015; Klepper & Herbert, 1991; Motts & Schofield, 2010, 2011; Peruzzi & Dut, 2004; Schofield & Motts, 2009). Inputs from other sensory domains are also relayed to the IC, especially to the lateral or external cortex (ECIC). However, it is less understood how contextual information relevant for detecting and acting on auditory signals reaches IC.

The cerebellum can be a source of contextual and arousal input to IC. Neurons in the cerebellar midvermis (lobules VI/VII) respond to teleceptive stimuli, including sounds (Deura & Snider, 1964; Huang & Liu, 1990; Huang, Liu, Yang, Mu, & Hsia, 1991; Snider & Stowell, 1944). The cerebellar midvermis also receives anatomical inputs from the IC via pontocerebellar mossy fibers (Aitkin & Boyd, 1978; Azizi et al., 1981; Mihailoff, Kosinski, Azizi, & Border, 1989). Intriguingly, electrical cerebellar vermis stimulation modulates acoustic responses throughout the auditory system (Crispino & Bullock, 1984; Velluti & Crispino, 1979). Functionally, the cerebellar vermis is important for making context-dependent behaviors and has known roles in the prediction of sensory consequences of movement, orienting, long-term modification of startle, and other non-motor functions, including arousal (Baumann et al., 2015a; Blakemore et al., 2001; Bodranghien et al., 2015; Koziol et al., 2014; Leaton & Supple, 1986; Steriade, 1995). Thus, the cerebellar vermis would be able to provide the IC with integrated, contextual signals for generating appropriate behaviors to relevant stimuli.

To study the functional connectivity of the cerebellar vermis to the IC, I optogenetically stimulated cerebellar output while recording extracellular activity in the IC of awake mice head-fixed on a treadmill. The majority of recorded IC units were robustly modulated by vermis photostimulation, with over half excited and a minority inhibited following light onset. These vermis responsive units are located throughout the IC, but had topographical biases in the distribution of response types. Results from this study indicate that the cerebellar vermis can directly drive activity in the IC and furthers our understanding of brain circuits that can help animals produce appropriate behavioral responses to different stimuli.

Methods and Materials

Animal care and usage

All the experimental procedures used in this research study were approved by the Johns Hopkins University Animal Care and Use Committee in accordance with National Institutes of Health guidelines. Mice (NOS1-ChR2, L7-cre; ai40, and L7-cre; ai27) were housed and bred in an onsite vivarium on a 12-h dark/12-h light cycle (dark from 18:00 to 06:00). For vermis optogenetic manipulation, I used NOS1-ChR2 bacterial artificial chromosome transgenic mice expressing ChR2 under the control of neuronal nitric oxide synthase (nNOS) promoter (Kim et al., 2014) as well as L7-cre;ai27 transgenic mice expressing archaerhodopsin in Purkinje cells. Mice of both genders were aged 8 – 16 weeks during the experiments, which were performed between 09:00 and 18:00.

Experiment Procedure Overview

First, mice were anesthetized and implanted with headpost. Post-surgery, they were allowed to recover for 1-3 days and acclimated on the cylindrical treadmill. Once the mice were able to move smoothly on the treadmill, a craniotomy was made over bilateral IC. Each mouse was recorded from daily with tungsten electrodes up to 2 hours a day. Data collection through Spike2 (Cambridge Electronic Design) simultaneously recorded spikes, local field potentials, treadmill movement, and stimulus trigger times. Electrolytic lesions were made at the ventral tips of most electrode tracts. Following the final recording session, mice were anesthetized (5% isoflurane by volume), perfused

(phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS), and brains were removed for sectioning and electrode track reconstruction.

Head-fixed animal preparation.

In preparation for neuronal recordings, mice were anaesthetized with isoflurane (1-2% by volume) and an acrylic headpost was implanted onto the skull (Kodama & du Lac, 2016). Mice were kept under chronic isoflurane anesthesia (1-2% by volume) throughout all surgeries. Mice were placed in a mouse adaptor which held the head stable with a palate bar and nose clamp. Ear bars were not used to prevent potential damage to the eardrum.

Petroleum jelly was applied to the mouse's eyes to keep them moisturized during the surgery. The mice were placed atop a heating pad throughout the surgery. The mouse's scalp was cleaned with 70% ethanol and hexidine, and then shaved with surgical scissors. A single incision was made along the scalp midline to expose the skull overlaying the cerebellum caudally and the forebrain rostrally. The skull overlaying cerebellar vermis was marked with Sharpie marker to denote the location of underlying vermis lobules, as indicated by the blood vessels visible through the skull, to help guide photostimulation targeting.

A thin layer of Metabond (Parkell) was applied to the entire exposed skull surface for increased skull integrity. Bone wax was applied to the skull above bilateral inferior colliculus and the cerebellar vermis. The acrylic headpost was then fixed to the skull with Metabond. A recording chamber (above bilateral inferior colliculus) and stimulation chamber (above bilateral cerebellar vermis) were made by applying Metabond around the

bone wax. Post-surgery, meloxicam (4 mg/kg) was injected subcutaneously before the mouse was returned to a separate cage for recovery.

After the recovery period, the mouse was acclimated to head fixation and walking on the cylindrical treadmill. To fix the mouse's head, the headpost was screwed tightly to a metal bar which was adjusted so that the mouse could move comfortably on the treadmill. The mice were allowed to locomote freely on the treadmill for up to an hour a day until they were able to run smoothly, typically within 2 days.

Before the start of electrophysiological recordings, the mouse was anesthetized with isoflurane (1-2% by volume) and a craniotomy was made over the IC region. The recording chamber and stimulation chamber were covered with a fast-curing silicone elastomer sealant (Kwik-Cast; World Precision Instruments) that could be removed before each experiment to give access to the brain. Care was taken to reduce bleeding and inflammation during the craniotomy surgery so that the dural surface would remain healthy for many days of recording.

In vivo *extracellular recordings in awake animals.*

Before each recording session, mice were lightly anesthetized with isoflurane (1% by volume) and head-fixed to the treadmill bar. The Kwik-Cast covering the recording and stimulation chambers were removed, and brain tissue health was inspected visually.

Unit and local field potential recordings were performed using tungsten electrodes (1-5 Mohms; FHC). The brain surface was kept moist with saline solution in the recording chamber, which was covered with Kwik-Cast for extra stability after electrode penetration. Neural signals were recorded with a Multiclamp 700B amplifier (Axon

Instruments) at a 2,000x gain and digitized at a 20,000 Hz sampling rate. Extracellular unit signals were band-pass filtered at 300 – 3,000 Hz while LFP signals were filtered at 1 – 300 Hz. The rotation speed of the treadmill was detected and recorded by a rotary optical recorder (US Digital) in real time and digitized at 2,000 Hz.

Sound and optogenetic stimulation.

Auditory clicks were calibrated and delivered through a speaker placed directly in front of the mouse. For the majority of recordings, the auditory click intensity was held at 70 dB SPL. The duration of the click was 0.5 s. During each experiment block, the click stimulus was repeated at least 30 times. Sound amplitudes were calibrated using a Sokolich Generation-II probe microphone with a sensitivity of 1000 mV/Pa at a frequency of 1 kHz.

For optogenetic stimulation, a blue LED light pulse was delivered through an optic fiber (ThorLabs; 550 μm core diameter). The peak intensity of the LED was either 113 mW/mm^2 or 210 mW/mm^2 (as measured at the tip of the optic fiber by photometer). For all experiments unless otherwise noted, the light pulse duration was 20 msec at peak intensity. For experiments testing the effects of photostimulation duration, blocks of 10, 20, 50, or 100 msec duration light pulses were used. Unless otherwise noted, both the auditory clicks and photostimulation were presented regularly every 1 s in separate blocks.

Vermis optogenetic stimulation preparation.

To optogenetically stimulate cerebellar vermis, an optic fiber (550 μm core diameter, Thorlabs) connected to a blue LED source (470 nm, Thorlabs) was placed on the skull surface above cerebellar vermis to transcranially stimulate the underlying neurons. Because the excitatory projections of cerebellar output nuclei neurons are primarily contralateral, the tip of the optic fiber was targeted above the lobule 6/7 vermis contralateral to the IC recording location. Vermal lobules 6/7 are associated with non-motoric functions as well as previously described auditory receiving areas (Snider & Stowell, 1944). The cerebellar cortex is relatively transparent (Al-Juboori et al., 2013), and I found that photostimulation through the thin skull was sufficient to evoke animal movement and IC activity.

For experiments using light for visual stimulation (Fig 2.5), well-isolated vermis-responsive units were recorded in the IC for >100 photostimulation trials. The optic fiber was then removed from the vermis skull surface and placed near the animal's eye (~ 10 cm away). Following light stimulation away from the cerebellum, the optic fiber was replaced to the original vermis stimulation site and evoked responses returned to ensure that the unit was not lost during fiber optic movement.

Mapping of recording locations.

Before recording, the tungsten electrodes were coated with a red fluorescent dye (DiI: 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate; ThermoFisher) to enable subsequent identification of electrode tracts locations. Electrolytic lesions were

made at the ventral end of a subset of electrode penetrations by passing 12mA current through an IsoFlex stimulus isolator (AMPI) for 20-30 s.

Following the final experiment session, mice were anesthetized with 5% isoflurane (by volume) and administered a terminal dose of Avertin (Sigma-Aldrich) and transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. Their brains were removed and transferred into 4% paraformaldehyde in PBS solution at 4°C for post-fixation overnight. The brains were then placed in 30% sucrose in PBS on a shaker until the brains sank to the bottom (approximately 12-24 hours) for cryoprotection. Thin coronal sections (40 – 60 μm) were cut with a freezing microtome through the midbrain. Sections were washed with PBS on a shaker and then mounted on microscope slides with either FluorSave (Millipore) or VectaShield (Vector Laboratories).

Images for each section were acquired using an Olympus BX61 fluorescence microscope. Images were collected using ImageJ (NIH) and then transferred to Adobe Illustrator. The positions of the electrode tracts were estimated by creating best-fit lines through the DiI markings across multiple sections. The depths of the recorded neurons were estimated based on both the micromanipulator reading and electrode tract reconstruction measurements. A normalized IC map was generated based off (Franklin & Paxinos, 2008) and only single units on reconstructed tracks (with visualized DiI and/or electrolytic lesions) were mapped (Fig 2.7).

Data analysis

Data analysis was performed with custom-written software and code in Igor Pro 7 (WaveMetrics). Spikes were sorted in Spike2 (Cambridge Electronic Design).

Trials were first manually masked for noise artifacts. Then stimulus triggered trials were separated according to animal movement. Trials were defined as stationary if the treadmill movement as measured by the rotary optical encoder did not exceed 10 mm/s in the 300 msec preceding stimulus presentation. If the treadmill exceeded 10 mm/s, the mouse was classified as running and those trials were analyzed separately (Chapter 4).

IC units were classified as excited by vermis photostimulation if the peristimulus time histogram (PSTH; 2 msec bin size) exceeded the spontaneous firing rate by 4 standard deviations of baseline fluctuation. The response onset latency was calculated as the timepoint on the PSTH that reached this threshold. For IC neurons that were inhibited, the response threshold and onset latency was calculated instead with 3 standard deviations. The Z-score was calculated by dividing the evoked response (calculated from a 300 msec window after stimulus onset) by the standard deviation of the baseline firing (calculated from a 100 msec window before stimulus onset). IC unit evoked responses to vermis photostimulation in NOS1-ChR2 and L7-cre; ArchT mice were indistinguishable, and combined for the analyses (Fig 2.5). A subset of IC units had both excited and inhibited responses (Fig 2.3) or response latencies outside the normal range (< 20 msec or > 150 msec), and were excluded from further analyses.

All values are presented as mean ± SEM unless otherwise noted. Methods described here were also used for experiments and analyses in Chapters 3 and 4.

Results

Brief cerebellar vermis photostimulation modulates inferior colliculus (IC) neural activity.

In order to investigate whether the cerebellum influences the neural activity in the inferior colliculus (IC), I performed extracellular recordings in the mouse inferior colliculus while optogenetically stimulating the cerebellar vermis. Because anesthesia is known to perturb brain activity, including in the auditory system and cerebellum (Alkire, Hudetz, & Tononi, 2008), I conducted these experiments *in vivo* using awake, head-fixed mice on a cylindrical treadmill (Fig 2.1).

Brief photostimulation of the cerebellar vermis (lobule VI/VII) in NOS1-ChR2 transgenic mice evoked robust neural activity in the inferior colliculus. Figure 2.2A shows the raw recording trace of a representative vermis-responsive IC neuron during photostimulation trials. Twenty msec light pulses delivered transcranially to the vermis reliably produce several action potentials in this IC single unit. This neuron had a spontaneous firing rate of 3.4 Hz and increased its neuronal firing starting at 37 msec from the onset of light stimulation (Fig 2.2B). The neuron's evoked response peaked at 188.5 Hz above its baseline and lasted 36 msec before returning back down to baseline. Figure 2.2B shows the raster plot (top) of the neuron's spiking activity each photostimulation trial, which was presented at a fixed 1 s intertrial interval. The peristimulus time histogram (PSTH; bottom) depicts the average firing rate of the neuron following vermis photostimulation (bin size of 2 msec). The vermis evoked response in this IC neuron was thus robust and repeatable across many trials.

Figure 2.2C depicts another example IC neuron recorded in a different NOS1-ChR2 animal that also exhibited an excited evoked response following vermis photostimulation. This cell had a response onset latency of 49 msec and an evoked firing rate peaking 80.7 Hz above its 18.2 Hz spontaneous firing rate. IC units were classified as excited by vermis photostimulation if the evoked activity exceeded 4 standard deviations of the baseline firing rate fluctuation (calculated from the 300 msec time window preceding light stimulation). The timepoint when the firing rate exceeded this threshold was calculated to determine the response onset latency (Fig. 2.2C).

Recording units throughout the IC, I observed several different types of responses to vermis photostimulation. In addition to cells with response latencies around 30 – 60 msec (Figs 2.2B, C), some IC neurons had longer response latencies; for example, the single unit in Figure 2.2D exhibited increased firing 95 msec after photostimulation with a smaller evoked peak response of 47.5 Hz above its baseline activity of 2.6 Hz. Other neurons had two peaks of evoked response, each of which corresponded to the shorter and longer response onset latencies. The cell in Figure 2.2D is a representative well-isolated single unit with a baseline firing rate of 4.2 Hz with one peak of evoked activity starting at 39 msec followed by a second peak starting at 68 msec.

Out of the 266 IC units I analyzed, over half (144/266 units, 54.1%) were excited by vermis stimulation (Fig 2.2H). I observed a population of IC units that were excited with an earlier response latency of 30 – 60 msec (Fig 2.2G, B, C), which accounted for 65/144 excited units. Another smaller population of IC units (39/144 excited units) exhibited a more delayed onset latency of > 80 msec and lower evoked firing rates (Fig 2.2G, D). A third population of IC units excited by vermis stimulation had two peaks of

activity that corresponded to the early and late response latencies (as in Fig 2.2D), making up the remaining 40/144 excited IC units. The population distribution in response latencies did not differ between single units and multiunit recordings (Fig 2.2F). A smaller subset of IC neurons (20/266 units; 7.5%) was inhibited by vermis photostimulation. The representative inhibited neuron in Figure 2.2F had a brief pause in firing lasting ~45 msec following vermis photostimulation. It should be noted, however, that the number of inhibited neurons may be undersampled due to low baseline firing rates in some IC neurons. Finally, a small number of IC units (21 single and multiunits) had a mixed/"weird" response that did not qualitatively fit with the purely excited or inhibited responses. Some of these units were both excited and inhibited (Fig 2.3A and B) while others had relatively weak responses at latencies that were outside the range of most robustly responsive units (< 20 msec or > 150 msec). Over a third of the IC units I recorded (98/266 units; 36.8%) did not respond to vermis photostimulation. There were no noticeable sex- or age-related differences in vermis evoked responses. In all, brief vermis photostimulation robustly modulated the majority of the IC units I recorded (Fig. 2.2H).

These data indicate that the cerebellar vermis is functionally connected with the inferior colliculus. However, there are no known direct anatomical connections between the cerebellum and the inferior colliculus, which I confirmed with retrograde tracer injections in IC and anterograde tracer injections into the cerebellar output nucleus of the vermis, the fastigial nucleus (FN). In addition, the response onset latency was relatively long for the vermis responsive IC units to respond via a direct monosynaptic connection from the fastigial nucleus. Thus, the circuit(s) functionally connecting the cerebellar

vermis and the IC are polysynaptic but evoke robust, consistent responses when optogenetically stimulated.

Vermis photostimulation duration and intensity affects IC modulation

To further investigate the effects of cerebellar stimulation on the IC, I tested different photostimulation durations and light intensity levels in a subset of vermis responsive IC units (n = 9 single units). Figure 2.4A shows an IC single unit that was robustly driven by 20 msec vermis photostimulation and then subjected to blocks of photostimulation of 10, 50, and 100 msec in duration. Shorter stimulation durations of 10 msec were not sufficient to produce evoked responses in this IC neuron, while longer 50 msec duration light stimulation evoked responses of similar magnitude and onset latency as with 20 msec duration photostimulation (Fig 2.4A, B). When the vermis was stimulated for 100 msec, however, the unit produced evoked responses which were smaller but had the same onset latency (Fig 2.4A, B). The consistent latency of IC response suggests that the onset of photostimulation was what drives cerebellar modulation of IC activity, and not the offset. In addition, the decreased IC evoked during 100 msec photostimulation suggests that prolonged stimulation of cerebellar inhibitory interneurons may produce more complex, potentially conflicting responses that reduces the overall cerebellar output reaching the IC.

Changes in photostimulation light intensity correspondingly changed the magnitude of IC evoked response. All the previously described results were conducted using a light intensity of 113 mW/mm^2 as measured from the tip of the optic fiber. As

expected, decreasing light intensity decreased the effect of vermis stimulation on IC activity (2.4C, D). Figure 2.4C shows the average evoked responses of an example IC neuron to varying light intensities. The response magnitude was similar at 67.8 mW/mm^2 and 113 mW/mm^2, but reduced at 45 and 23 mW/mm^2. This pattern held true across 13 units subject to the same photostimulation intensity testing (Fig. 2.4D). Because the optic fiber was located on the surface of the skull above the cerebellum, increasing light intensity was likely to activate larger populations of NOS1-expressing neurons due to increased light penetrance through the skull and underlying brain tissue (Al-Juboori et al., 2013). The lowest light intensities used were probably not sufficient to penetrate the skull surface and/or activate enough neurons to generate a sufficient cerebellar output signal.

Light stimulation of the visual system does not evoke IC activity in vermis-responsive neurons

IC neurons in monkeys were previously reported to respond to visual-related activity (Bulkin & Groh, 2012; Gruters & Groh, 2012b; Porter et al., 2007) so it is possible that the blue light pulses used for cerebellar photostimulation were driving the observed evoked responses through the visual system. To address this possibility, during recording of vermis-responsive units in three different animals I removed the optic fiber from the cerebellar surface and directed it near the animal's eye to activate the visual system, but not the cerebellum. I found that light pulses of the same intensity and duration activating the visual system, as opposed to the cerebellum, did not induce IC

activity (Fig. 2.5). In addition, for one animal, I placed the optic fiber on the surface of contralateral IC, to control for the possibility of light leakage through the brain tissue to the mouse's retina, and found that light pulses delivered there also did not evoke activity in a vermis-responsive IC neuron (not shown). Thus, modulation of IC activity was driven cerebellar stimulation, and did not result from a visual system response to the light pulse.

Direct Purkinje cell optogenetic photoinhibition directly drives IC evoked responses

The majority of experiments were conducted in NOS1-ChR1 transgenic mice, in which optogenetic stimulation indirectly inhibits Purkinje cells by exciting inhibitory interneurons in the molecular layer of cerebellar cortex. For a subset of experiments, I directly inhibited PCs by photostimulating the vermis in L7-cre; ai40 transgenic mice, which express the light-driven photon pump, archaerhodopsin (ArchT) selectively in PCs. In these mice, brief light pulses to cerebellar vermis directly inhibit PC activity. Photostimulation of the vermis in three ArchT mice also evoked IC activity similar to the modulation observed in experiments conducted using NOS1-ChR2 mice (Fig. 2.5A). Of the 21 IC units I analyzed in ArchT mice, the average maximum evoked firing rate was 50.18 ± 8.30 Hz with an average response onset latency of 43.89 ± 1.61 msec (Fig 2.5B).

Because the evoked activity in IC was not significantly different from experiments conducted in NOS1-ChR2 mice, results from both mouse lines were combined in my analyses (Fig 2.2G). Confirming cerebellar modulation of IC neural activity with two different transgenic mouse lines provides converging lines of evidence

that Purkinje cells inhibition, whether optogenetically coordinated directly or indirectly, is sufficient to influence activity in the IC.

Purkinje cell excitation does not directly drive IC response

Given the discovery that IC neurons are modulated by PC inhibition, I also investigated whether excitation of PCs exerts the opposite effect. I leveraged the transgenic mouse line L7-cre; ai27, which specifically expresses channelrhodopsin in Purkinje cells and allows for direct excitation of PCs with photostimulation. Using the same experimental set-up as described previously (Fig 2.1), I recorded from IC neurons while optogenetically exciting PCs and found that PC excitation did not produce the opposite response in IC as PC inhibition. Instead, I discovered that IC neurons had evoked responses but with shifted latencies of onset compared to when the PCs were inhibited (not shown).

I hypothesize that the delayed evoked response is due to the suppression of PC activity following optogenetic excitation, which could increase cerebellar (fastigial) nuclei neuron activity and drive increased output similar to what occurs during PC inhibition, as described in electrophysiology experiments conducted by a research associate in the lab, Takashi Kodama. The increased fastigial nucleus activity resulting after PC excitation is potentially what drives IC activity, albeit at a delayed interval.

Different types of vermis responsive neurons are distributed topographically in the IC

Because there are functional and connectivity differences among the different subdivisions of the IC (Ito, Bishop, & Oliver, 2016; Ono & Ito, 2015; Winer & Schreiner, 2005), I next examined whether neurons responsive to vermis photostimulation were found in different regions of the IC. The central nucleus of the inferior colliculus (ICC) primarily receives ascending auditory input from brainstem auditory nuclei (Kudo & Niimi, 1980; Winer & Schreiner, 2005). The inferior colliculus shell, comprised of the external cortex (ECIC) and dorsal cortex (DCIC), receive the majority of top-down input from the auditory cortices (Ito et al., 2016; Ono & Ito, 2015; Winer & Schreiner, 2005). The ECIC also receives inputs from other sensory domains, including somatosensorial and eye position information (Aitkin et al., 1981; Gruters & Groh, 2012a; Lesicko & Llano, 2019). Thus, the distribution of vermis responsive units could provide insights into the IC circuits and functions that the cerebellum contributes to.

To map the location of recorded units, I coated the tungsten electrodes used for extracellular recording with a fluorescent dye (DiI) and made electrolytic lesions at the ventral tip of a subset of recording electrode tracks to reconstruct the electrode tracks. The depth measurement of the micromanipulator was also recorded for each recording location. Following the final recording session, mice were perfused and their brains sectioned and then imaged with an epifluorescence microscope to reconstruct the locations of the electrode tracts and provide estimates of each recording location. Using this methodology, I was able to reconstruct recording locations in approximately half of the electrode tracts in my dataset. Figure 2.7A depicts an example electrolytic lesion

made in the IC at the end of a recording surrounded by fluorescent DiI that was applied to the electrode.

I plotted the locations of single IC units in well reconstructed electrode tracts onto a normalized map of the IC and found that, while vermis responsive units were found distributed throughout the IC, units with different types of responses to vermis stimulation were clustered in different subdivisions (Fig 2.7B and Table 2.1). Two peak or long latency evoked neurons were found in laterally in the external cortex (ECIC); 10/14 of cells with two peak responses and 6/9 neurons with only late evoked activity were found in the ECIC (Fig 2.7B and Table 2.1). By contrast, neurons with an early peak of evoked firing were found more medially in either the dorsal cortex of IC (DCIC; 6/17 early peak neurons) or the central nucleus (CIC; 9/17 early peak neurons). Neurons inhibited by vermis photostimulation were located more ventrally in the IC, mostly in the CIC (6/7 inhibited neurons). Thus, the functional modulation of vermis on IC neurons was both widespread and topographically distributed in types of responses. The topography of response types could help inform hypotheses about the function cerebellar vermis plays in the IC (see Chapters 3 and 4).

IC responses to auditory clicks were heterogenous and similar to their responses to vermis stimulation

IC neurons have heterogenous responses to auditory stimuli (Aitkin, Tran, & Syka, 1994; Malmierca, Cristaudo, Pérez-González, & Covey, 2009; Rees, Sarbaz, Malmierca, & Le Beau, 1997; Schreiner & Langner, 1988; Syka, Popelár, Kvasnák, &

Astl, 2000). I examined whether the responses of IC neurons to vermis stimulation corresponded to their auditory responses. During each experiment session, I presented the animal with blocks of auditory clicks trials interspersed with blocks of vermis stimulation trials. IC units that were excited by vermis stimulation exhibited a variety of responses to a standardized 70 dB SPL auditory click delivered every 1 s through a speaker placed directly in front of the animal (Fig 2.8). Figures 2.8A, B, and C shows the corresponding auditory click responses (on the right) in three different vermis-responsive IC neurons (left). Though all three cells had an early evoked response following vermis photostimulation, the neuron in 2.8A had a sustained response following the auditory click lasting 90 msec, while the cell in 2.8B exhibited a more phasic response lasting 30 msec. The cell in 1.8C exhibited two peaks of excitatory response to auditory click. Across 55 single IC units excited by vermis photostimulation, the evoked click responses tended to be larger than vermis evoked responses (Fig 2.8D). There was no clear correlation between the response latencies of vermis and click evoked responses (Fig 2.8E).

Interestingly, the IC units inhibited by vermis photostimulation were also consistently inhibited by auditory click (Fig 2.9). Figure 2.9A shows a representative vermis-inhibited IC neuron that had a reliable pause in neural activity following a 70 dB SPL click. The duration of the pause in firing lasted for similar durations following vermis photostimulation and auditory click (~50 msec). Across the population of 10 inhibited single units I analyzed, the magnitude of inhibition following auditory click corresponded with the inhibition from vermis photostimulation (Fig. 2.9B). These results

suggest that the is, at least in part, co-opts the same circuitry that relays auditory click information to the IC.

The same recording locations IC can have heterogenous responses to vermis and auditory stimulation

Underscoring the functional heterogeneity of the IC, in some recordings multiple separable units were recorded exhibiting different response types to auditory click and vermis photostimulation at the same recording location (Fig 2.10). Figure 2.10A depicts an example recording site with two neurons that responded differently to the same vermis photostimulation and auditory click trials. The neuron on top had two peaks of activity following vermis photostimulation and a sustained response to auditory clicks. In contrast, the neuron on the bottom had a weaker delayed response following vermis photostimulation and no response to auditory clicks. From my reconstruction and subsequent mapping, these neurons were located in the ECIC.

Two different neurons from a second example recording are is shown in Figure 2.10B. Here, the top neuron had a long latency response (95 msec) to vermis photostimulation with a robust and delayed response latency (17 msec) to auditory click. The bottom neuron had no response to cerebellar stimulation and a weak, phasic response to auditory click.

These examples highlight the heterogeneity of neural responses in IC, and also underscores that location and auditory responsiveness alone are not sufficient to predict whether a particular IC neuron would be modulated by vermis stimulation.

Discussion

In this study, I optogenetically stimulated PCs in the cerebellar midvermis while extracellularly recording neurons in the IC of awake mice head-fixed on a treadmill. Brief suppressions of PC activity robustly modulate the majority of neurons throughout the IC. Neurons excited by vermis photostimulation had peaks of activity occurring early (30 – 60 msec from stimulation onset), late (after 100 msec), or both early and late. A minority of cells were also inhibited by vermis photostimulation. Neurons with vermis-evoked two-peaked responses or late responses were typically found laterally in the ECIC, while early responses were found more medially in the DCIC or CIC. Neurons inhibited by vermis photostimulation were preferentially located deeper in the CIC. However, different response types to auditory clicks and vermis photostimulation could be found in the same recording location. In addition, neurons inhibited by vermis photostimulation were also inhibited by auditory clicks.

The majority of IC neurons are modulated by optogenetic stimulation of cerebellar vermis

Previous studies implicated the cerebellum in modulating activity in the auditory system. In particular, electrical stimulation of the midvermis cerebellar cortex (lobules V

– VII) was found most effective in attenuating the auditory brainstem response (ABR) following auditory clicks (Crispino & Bullock, 1984). The IC sends projections to the dorsolateral pontine nucleus, which in turn sends ascending mossy fiber inputs to the midvermis (Azizi et al., 1985, 1981; Huffman & Henson, 1990). Responses to auditory stimuli were recorded reliably in different cell types of this region of the vermis (Snider & Stowell, 1944).

In this study, I optogenetically stimulated lobule VII of the cerebellar vermis while recording extracellular activity in the IC. Previous studies investigating cerebellar effects on the auditory system were conducted using electrical stimulation of the vermis, which stimulates many different cell types as well as potentially affects other brain areas by exciting fibers of passage at the site of stimulation (Crispino & Bullock, 1984; Velluti & Crispino, 1979). The optogenetic approach employed here allows for cleaner manipulation with cell-type specificity.

I optogenetically inhibited PC activity both directly and indirectly using two different transgenic mouse lines. NOS1-ChR2 BAC transgenic mice express channelrhodopsin-2 selectively in gap junction-coupled molecular layer inhibitory interneurons, which converge upon and inhibit PCs (Kim et al., 2014). Photostimulation of the vermis in NOS1-ChR2 mice robustly inhibits PC activity (Kim et al., 2014; Fig 1.1), in turn disinhibiting fastigial nucleus (FN) neurons that project to various downstream targets in the rest of the brain (Fig 2.1). L7-cre; ArchT mice expresses the light-activated proton-pump, archaerhodopsin, selectively in PCs; in these mice, light stimulation directly inhibits PC activity. IC neurons are robustly modulated by vermis photostimulation in mice from both transgenic lines, indicating that PC inhibition,

leading to increased downstream FN activity, is likely what drives cerebellar output to the IC and the rest of the brain (Fig 2.2, 2.5). Increasing durations of photostimulation did not change the response latency of evoked responses in the IC, indicating that the onset of PC inhibition (and presumably the onset of increased FN activity) is what drove the response in IC (Fig 2.4).

The majority of recorded IC units were robustly excited by vermis photostimulation, with a few that were inhibited (Fig 2.2). The results in this study show that cerebellar vermis manipulation can alter neural activity in the auditory system in the absence of sound presentation, whereas previous studies showed vermis stimulation modulates responses to auditory stimuli (Crispino & Bullock, 1984; Velluti & Crispino, 1979).

Technical considerations of vermis photostimulation

There are several considerations for the stimulation parameters I used in testing vermis effects on IC neurons. Though optogenetic stimulation permits cell-type specificity in manipulation of neural activity, the extent and population of PCs affected are less precise.

The optic fiber used for photostimulation was placed on the skull above the vermis lobule VII contralateral to the IC recording electrode. Though the skull is thin (permitting visual identification of underlying blood vessels and brain tissue), it is uncertain how much of light penetrates and scatters throughout the vermis, and thus what proportion of PCs are recruited.

ChR2 requires a light power of 8-12 mW/mm^2 to be activated (Boyden, Zhang, Bamberg, Nagel, & Deisseroth, 2005), and the optic fibers I used had outputs of 113 to 210 mW/mm^2. The cerebellar cortex is more transparent than other brain regions in mice, and an estimated 28 mW of light intensity would be required to illuminate and activate ChR2 to a depth of 600 μm (Al-Juboori et al., 2013). With my stimulation intensity, it is likely that at least 600 μm of cerebellar vermis had sufficient light intensity to activate ChR2. When light intensities were reduced to 20 or 40% of the maximum, evoked responses in IC were substantially reduced as well (Fig 2.4C, D). Because light penetrance through tissue depth changes nonlinearly, this reduction in light intensity likely led to a dramatic decrease in the depth of photostimulation and thus the number of PCs indirectly inhibited.

Photostimulation was targeted to vermis lobule VII due to its known involvement in auditory responses, orienting behaviors, and neuropsychiatric disorders (Baumann et al., 2015a; Schmahmann, Weilburg, & Sherman, 2007; Snider & Stowell, 1944). The majority of PCs in lobule VII project to the oculomotor caudal FN, which makes specific projections to neuromodulatory brain areas and thalamocortical circuits that differ from outputs by other FN modules (Katoh, Arai, & Benedek, 2000; Noda, Sugita, & Ikeda, 1990; Voogd & Barmack, 2005; Zhang et al., 2016). However, the connectivity of PCs to the FN is organized by parasagittal, aldolase C (zebrin II)-positive and negative stripes that traverse the different lobules of the cerebellar cortex and correspond to different compartments of cerebellar output nuclei, including the FN (Apps & Hawkes, 2009; Apps et al., 2018; Chung, Marzban, & Hawkes, 2009; Sugihara, 2011; Sugihara & Shinoda, 2004, 2007; Zhou, Lin, et al., 2014). In particular, aldolase C-positive PCs in

the vermis project to the caudal FN, while aldolase C -negative PCs project to the rostral portion (Sugihara, 2011). There is a zebrin negative stripe that is in the vermis midline projecting to rostral FN (Sugihara & Shinoda, 2004), which photostimulation of vermal lobule VII would also likely affect. Thus, although the majority of PCs inactivated by optogenetic stimulation disinhibited neurons in the caudal FN, it is likely that rostral FN output was also disinhibited.

Finally, optogenetic stimulation requires the use of light pulses that, as a sensory stimulus, in itself could activate the IC. The IC is reported to receive inputs from visual areas, including the primary visual cortex, superior colliculus, and the retina (Bulkin & Groh, 2012; Gruters & Groh, 2012a; Porter et al., 2007). Visual or saccade-related responses were reported in 42-64% of recorded IC units in monkeys with similar latencies of response as vermis evoked responses measured in this study, averaging about 70 msec after LED onset (Bulkin & Groh, 2012; Porter et al., 2007). Though my LED stimulation intensity was stronger than the light stimuli previous studies used, I did not observe any visual responses in the IC in cells that were modulated by vermis stimulation (Fig 2.5). There are several differences between my experiments and previous studies which may account for why I do not observe visual responses.

Visual stimuli were presented in the context of a behavioral task that required eye fixation, while in my experiments, light flashes were presented to the eye without a behavioral context that requires a trained output behavior (Bulkin & Groh, 2012; Gruters & Groh, 2012a; Porter et al., 2007). These previous studies were also conducted in primates, which are more visually-reliant animals than mice. It is possible that there is less or weaker visual input in the IC of mice than rhesus monkeys.

Different vermis response distributions in IC subdivisions may underlie different functions

Vermis responsive neurons were found in throughout the IC, but there was a biased distribution of response types in different anatomical subdivisions (Fig 2.7B). These subdivisions have different connectivity, which may help determine the influence vermal function has on the IC.

The central nucleus of the IC (CIC) primarily carries the ascending auditory signals from the periphery, while the shell region, which is comprised of the external (or lateral) cortex (ECIC) and dorsal cortex (DCIC) receive stronger non-auditory, top-down cortical, and neuromodulatory inputs (Winer & Schreiner, 2005). The shell regions, particularly ECIC, receive somatosensory, visual, and motor-related signals, which could integrate contextual information about the animal's bodily state (Gruters & Groh, 2012a; Zhou & Shore, 2006). In addition, the ECIC is part of an acousticomotor system that makes projections to the superior colliculus and the pontine nuclei inputs projecting to the cerebellar midvermis, which coordinate orienting behaviors (Azizi et al., 1985; Huffman & Henson, 1990). The ECIC, in particular, is involved in acoustic startle responses, and may be generally involved in alerting and orienting behaviors in response to different stimuli (Huffman & Henson, 1990).

Neurons with two peaks or late excited responses to vermis photostimulation were found predominantly in the ECIC, while early responses were found more medially in the DCIC or CIC (Fig 2.7B). Neurons that were inhibited by vermis photostimulation were located more ventral in the CIC (Fig. 2.7B). However, there is strong interconnectivity between different subdivisions of the IC, which could explain why vermal responsive

cells were found throughout the structure as well as why the same recording location in the IC had neurons with different response types to both vermis photostimulation auditory click (Fig 2.10).

In order to infer the functions of vermal input to the IC, I will discuss the relation of these evoked neural responses with the movements that vermis photostimulation and auditory clicks produces in head-fixed mice in Chapter 3.

The circuit mediating cerebellar input to the IC may partially co-opt the auditory circuit

Examining the response properties of IC neurons would help further differentiate the different types of neurons in relation to vermis-responding cells. The IC has heterogeneous responses to auditory clicks (Fig 2.8, 2.9), as well as other auditory stimuli (Hernández, Espinosa, Pérez-González, & Malmierca, 2005; Syka et al., 2000). Neurons with excited vermis evoked responses exhibited a variety of responses to auditory clicks (Fig 2.8), but neurons inhibited by vermis photostimulation were also inhibited by auditory clicks (Fig 2.9). This finding implies that the inhibited neurons may share the same neural inputs to the IC. Reconstructions of locations of inhibited IC units reveal that they were located ventrally in the CIC (Fig 2.7).

The relationship between neurons excited by vermis photostimulation and their acoustic click responses was less clear (Fig 2.8). However, the auditory stimuli presented were clicks, which could produce complex excitation and inhibitory responses throughout the auditory system. Testing the auditory responses of ICs to noisebursts or different tones may be more help parse out the types of auditory responses in relation to vermal response types. Previous studies on the auditory properties of the IC find that tone-

responsive neurons are found predominantly in the CIC, with neurons in the ECIC and DCIC more responsive to broadband noisebursts or vocalizations (Aitkin et al., 1994; Bulkin & Groh, 2011; Syka et al., 2000). In addition, there are neurons in the shell region and, to a lesser extent, in the CIC that do not respond to tested auditory stimuli (Aitkin et al., 1994). The IC is involved in sound localization, so varying the directionality of acoustic input may also help identify different cell types in the IC.

Figures

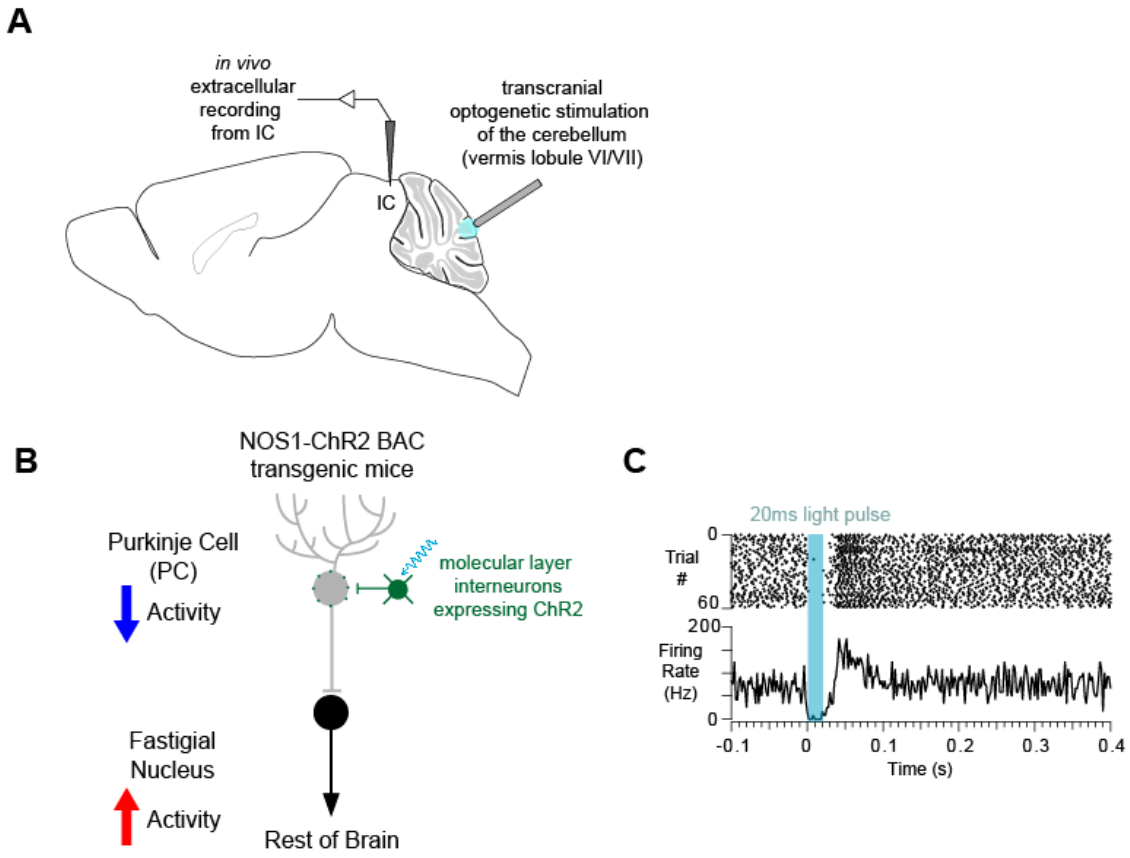


Figure 2.1. Experimental approach

A. Schematic of *in vivo* extracellular recording of IC in awake, head-fixed mice with vermis optogenetic stimulation. Mice were head-fixed on a cylindrical treadmill for all recordings.

B. Optogenetic photostimulation strategy for increasing cerebellar output via indirectly inhibiting Purkinje cell activity using NOS1-ChR2 activation of molecular layer inhibitory interneurons.

C. Representative Purkinje cell activity to brief photostimulation, recorded by Dr. Takashi Kodama.

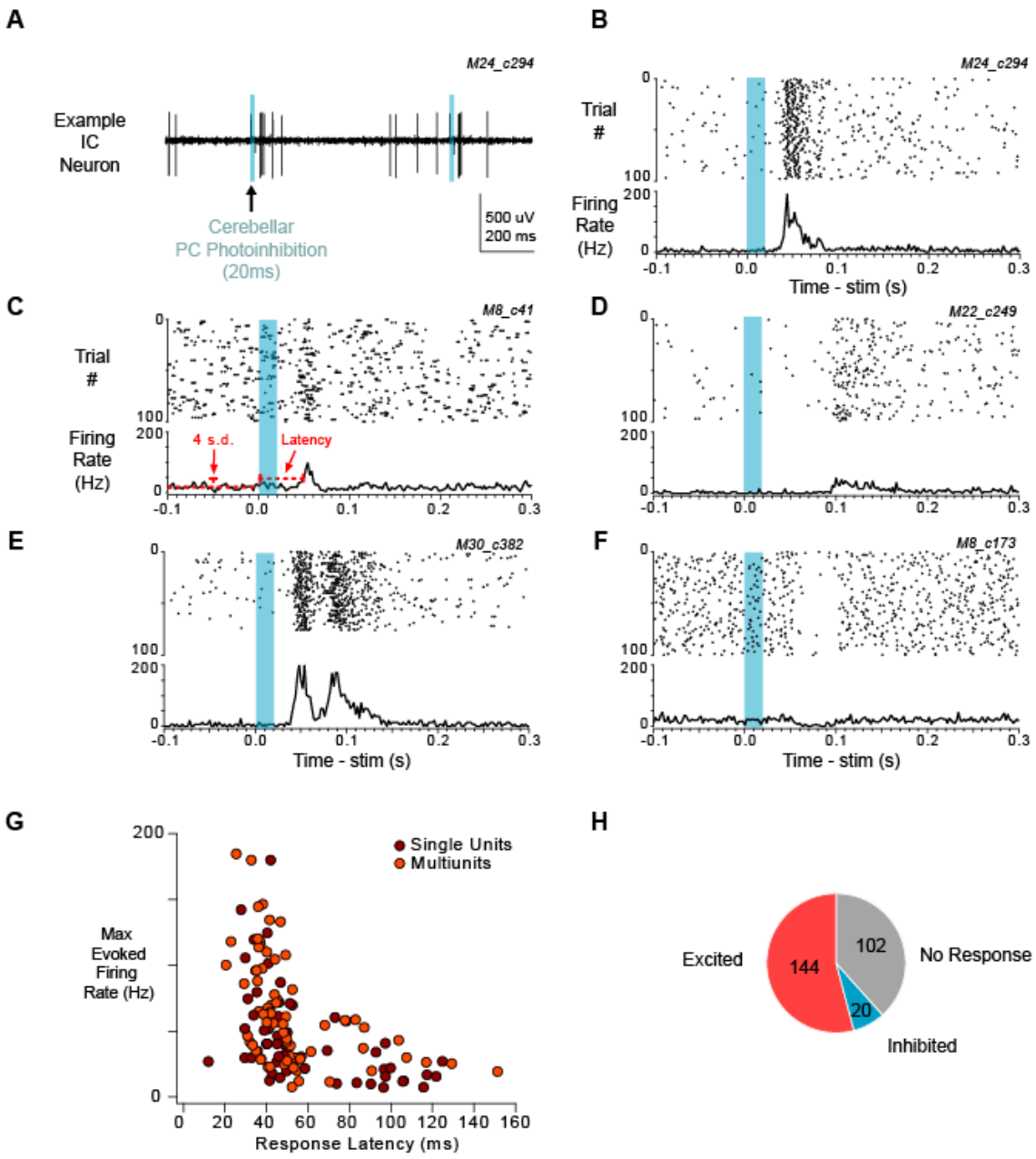


Figure 2.2: Cerebellar vermis PC inhibition evokes robust IC responses

A. Raw trace recording of representative IC neuron following brief cerebellar photostimulation. Representative IC neurons responsive to vermis photostimulation that were: B, Early excited. C, Late excited. D, Two peak excited. E, Inhibited.

F. Distribution of evoked response magnitude and latencies of excited IC units (n = 144 units).

G. Proportions of vermis photostimulation response types of units recorded in IC (n = 266 units).

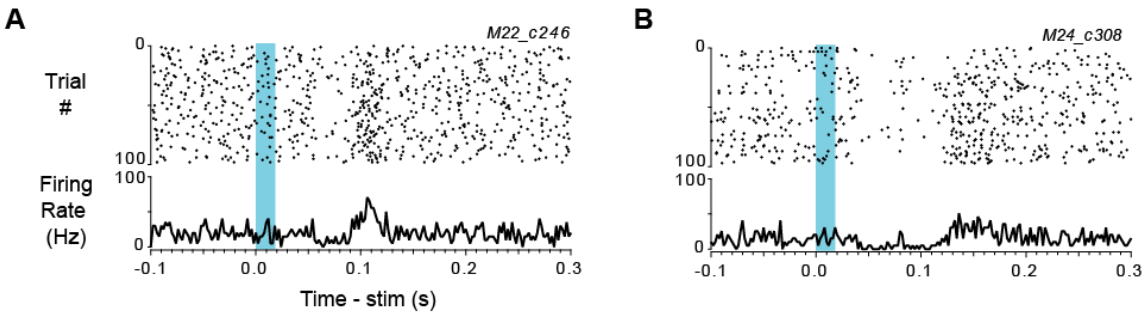


Figure 2.3: Cerebellar vermis PC inhibition can evoke mixed responses.

A, B. Example IC units with both excited and inhibited responses following vermis photostimulation.

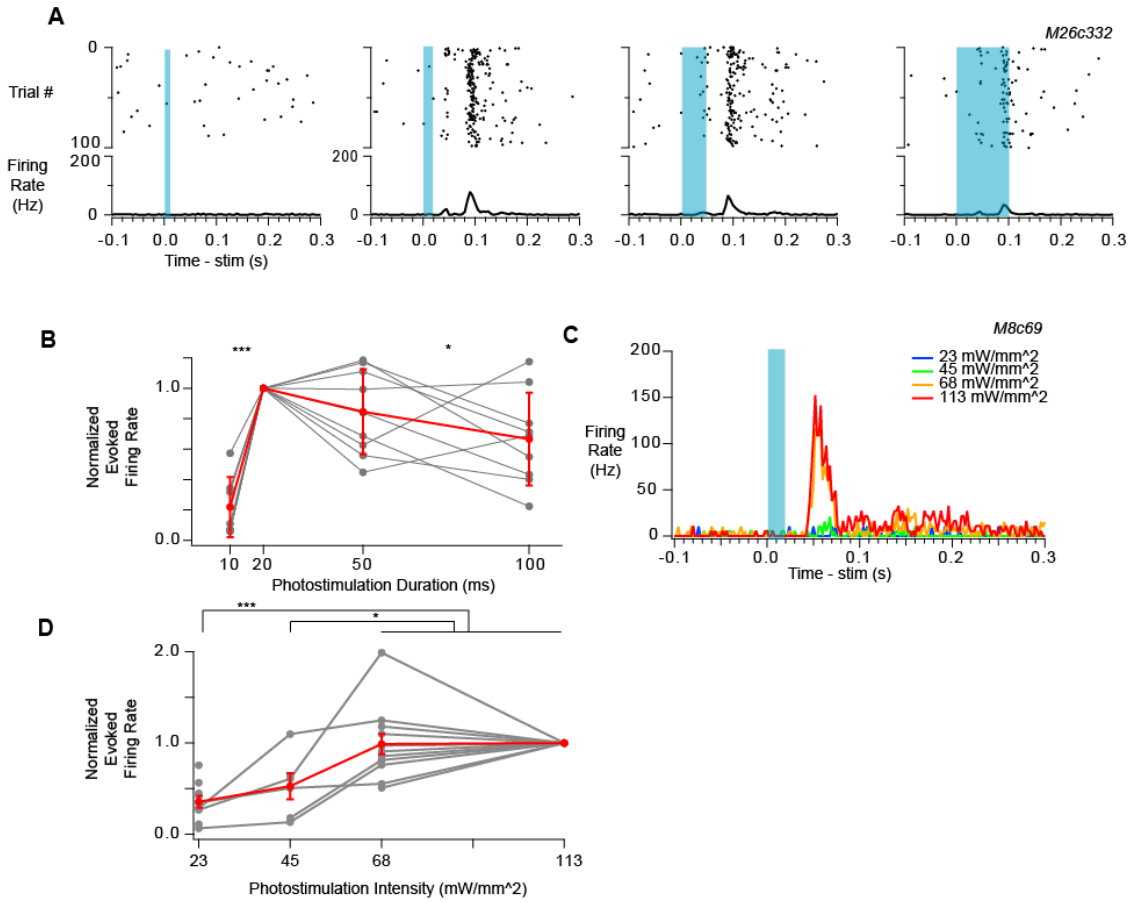


Figure 2.4: Parametric effects of vermis photostimulation on IC evoked activity

A. Example IC unit response to different durations of vermis photostimulation (10, 20, 50, and 100 msec).

B. Grouped IC max evoked response to different vermis photostimulation durations normalized to evoked response to 20 msec photostimulation duration (n = 9).

C. Representative IC unit evoked response to different intensities of photostimulation.

D. Grouped max evoked response to different photostimulation intensity (n = 13 units). Mean ± sem. *P<0.05; ***P < 0.0001.

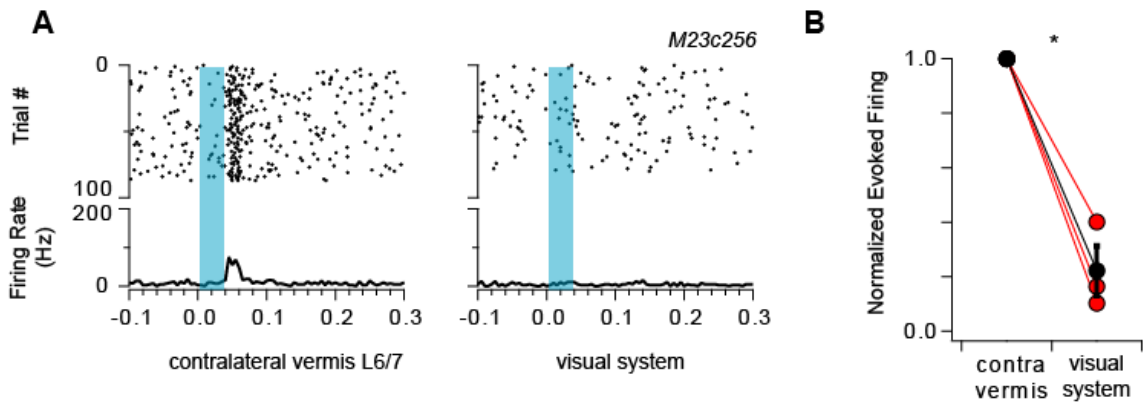


Figure 2.5: IC evoked activity driven by vermis modulation, not from visual stimulation

A. Example raster responses of cerebellar responsive IC unit to vermis stimulation versus light pulses near eye.

B. Normalized evoked responses of IC single units to contralateral vermis versus stimulation of the visual system (n = 3 mice). *P < 0.05 (two-tailed paired Wilcoxon Ranked Sign Test).

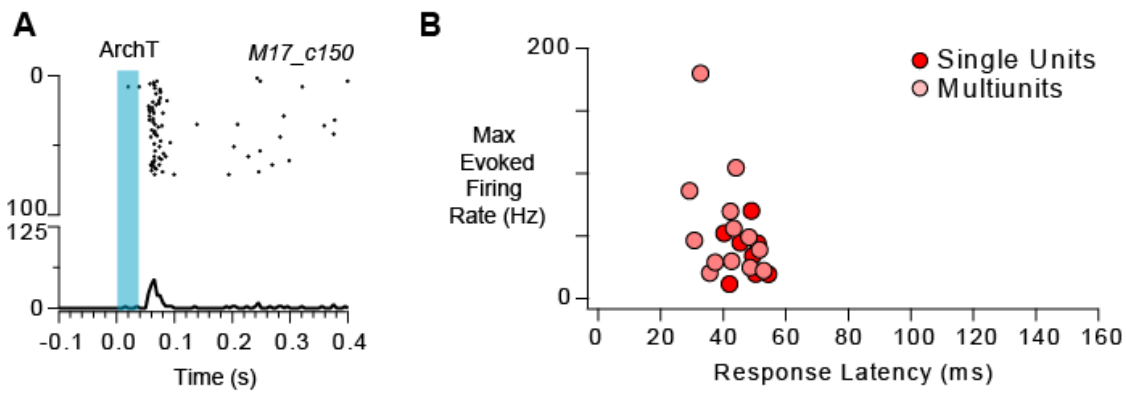


Figure 2.6: Direct PC inhibition produced similar evoked IC responses as with NOS1-ChR2 photostimulation.

A. Representative IC neuron evoked response from L7-cre; ArchT PC inhibition.

B. Group responses of ArchT evoked activity in IC (n = 21 units).

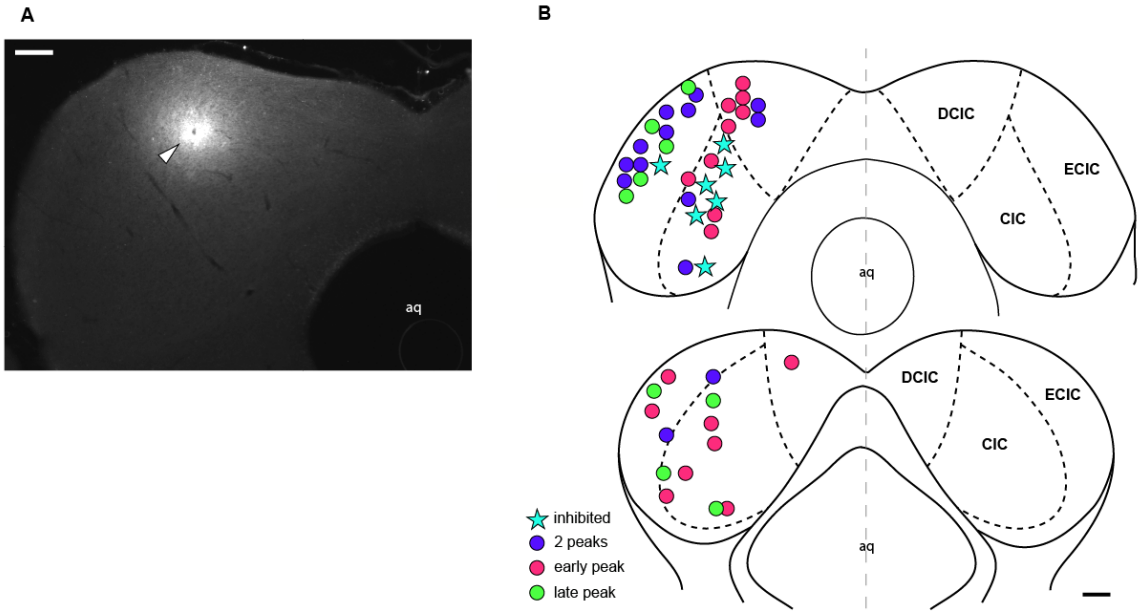


Figure 2.7: Different types of vermis responsive neurons were distributed topographically throughout the IC.

A. Example reconstruction of electrode tract marked with fluorescent dye (DiI). Arrowhead, site of electrolytic lesion.

B. Mapping of different vermis photostimulation response types of reconstructed subset of IC single units. Normalized schematic showing collapsed rostral (top) and caudal (bottom) IC adapted from The Mouse Brain Atlas (Franklin & Paxinos, 2008) (n = 47 single units).

Aq, cerebral aqueduct; CIC, central nucleus of IC; ECIC, external cortex of IC; DCIC, dorsal cortex of IC. Scale bars, 200 µm.

		Location in IC			
		ECIC	**DCIC**	**CIC**	**Total**
Response Type	**Inhibited**	1	0	6	7
	2 Peak	10	2	2	14
	Early Peak	2	6	9	17
	Late Peak	6	0	3	9
	Total	19	8	20	47

Table 2.1. Proportions of response types to vermis photostimulation in different subdivisions of IC.

Based on mapping estimates in Figure 2.7B.

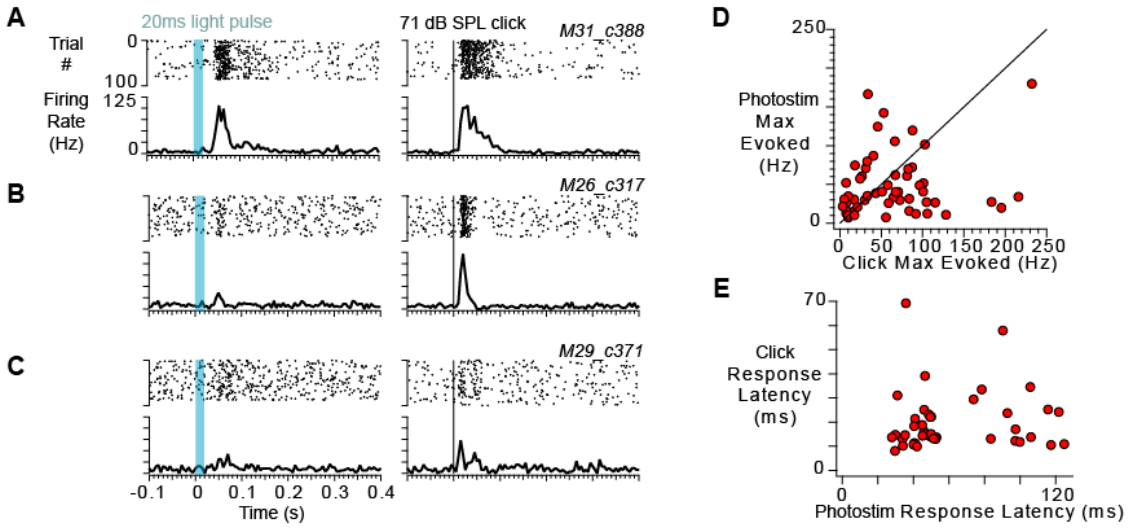


Figure 2.8: Auditory click responses were heterogenous and similar to excitatory vermis photostimulation responses

A, B, C. PSTHs of three example vermis-responsive IC neurons during photostimulation (left) and auditory click (right) trials.

D. Comparison of evoked responses of vermis excited neurons to photostimulation and auditory clicks (n = 55 single vermis evoked units).

E. Comparison of response latencies of single units to auditory click and photostimulation (n = 55 single vermis evoked units).

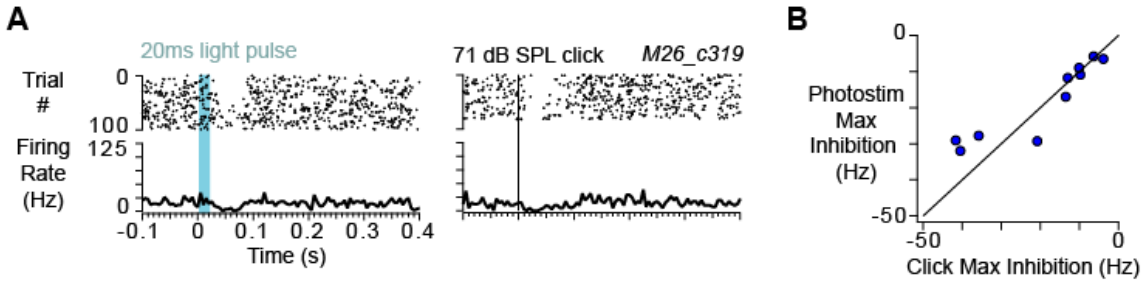


Figure 2.9: IC neurons inhibited by vermis photostimulation were also inhibited by auditory clicks

A. Representative IC neuron that was both inhibited by vermis photostimulation (left) and auditory click (right).

B. IC units inhibited by vermis photostimulation were consistently inhibited by auditory clicks as well (n = 10 IC neurons).

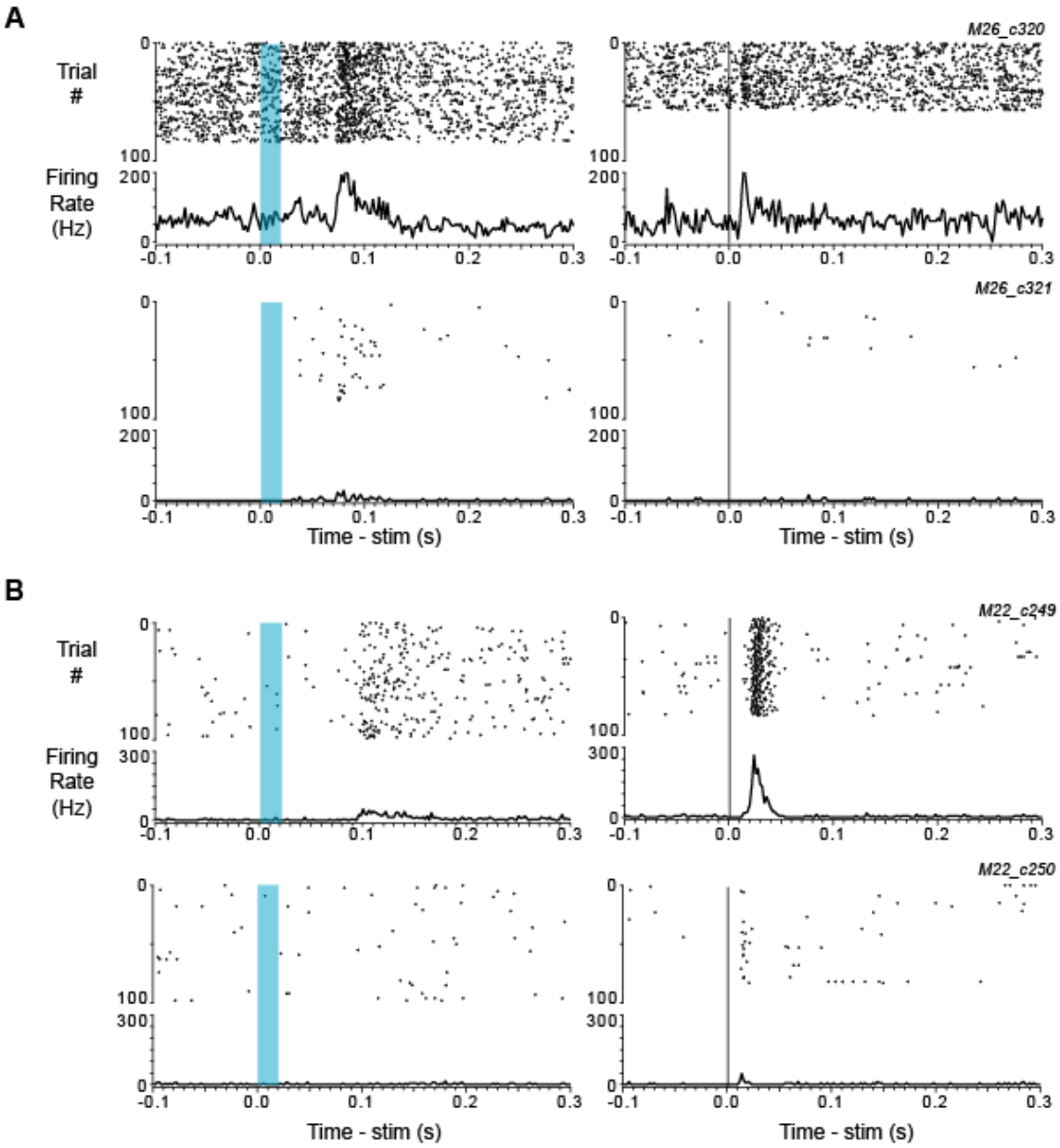


Figure 2.10: Same recording locations in IC can have different vermis and auditory responses

A. Single units (top and bottom) recorded at the same recording location during vermis photostimulation (left) and auditory click trials (right).

B. Different recording location with two different single units. Same convention as in (A).

Chapter 3. Motor-related activity in inferior colliculus neurons

Introduction

The cerebellum is classically considered a motor structure, associated with movement coordination, balance, posture, and oculomotor functions (Kheradmand & Zee, 2011; Matos, Fernanda, Veloz, Ruigrok, & Zeeuw, 2016). Studies by Bower, however, suggest that the cerebellum also plays a role in sensorimotor modulation (Baumann et al., 2015b; Bower & Kassel, 1990). Stimulation of the cerebellum can evoke movements. Electrical stimulation of lobule VI/VII, oculomotor vermis, drives saccades in monkeys (Noda & Fujikado, 1987).

More recently, converging lines of evidence from clinical, neuroimaging, anatomical, and behavioral studies highlight different roles that the cerebellum plays in non-motor functions (Baumann et al., 2015b; Bodranghien et al., 2015; Kelly & Strick, 2003; Middleton & Strick, 2002; Schmahmann & Caplan, 2006; Strick, Dum, & Fiez, 2009).

During my vermis photostimulation experiments (as described in Chapter 2), I observed that mice made brief, twitch-like movements in some stimulation trials. Given the role the cerebellum plays in motor function, I sought to understand what these evoked movements might be, and whether and how they relate to IC neural responses.

In addition to being a central hub in the auditory system, the inferior colliculus (IC) is involved in acousticomotor behaviors, and has saccade-related signals (Bulkin & Groh, 2012; Huffman & Henson, 1990; Porter et al., 2007). Given these motor-related

activities, it is possible that brief animal movement can modulate neural responses in the IC.

In these studies, I first analyzed the movements that head-fixed mice produced on the treadmill in response to loud auditory clicks that were presented irregularly or regularly, and found that mice produced a measurable short-latency startle movement followed by a longer latency movement. I next compared the auditory click evoked movements with the movements produced by vermis photostimulation, and found that they were similar. Analyzing vermis evoked firing in IC, I found that the early latency peak was dissociable from the early latency movement and had increased response probability in trials with late movement. By contrast, the late latency IC firing was time-locked to and correlated with late movement. In addition, some auditory click trials produced movement that also evoked firing. These results provide a comparison of IC responses with the movements made in reaction to salient stimuli.

Methods and Materials

Animal surgeries, general experimental procedures, and data analyses methods are the same as described in Chapter 2 with some additional methods below. Data collection of treadmill movement occurred simultaneously with neural data collection.

All recording experiments were conducted using head-fixed mice place atop of a cylindrical treadmill (Fig 3.1A). The treadmill was outfitted with a rotary optical encoder (ROE) (US Digital) to measure and record the velocity and direction of movement during recording experiments (Chettih, McDougle, Ruffolo, & Medina, 2011; Heiney, Kim,

Augustine, & Medina, 2014). The movement traces were offset so that the average treadmill velocity at the stimulus onset was 0 mm/s.

To test the behavioral response to actual startling stimuli, 100 dB SPL auditory clicks were presented at either 1 Hz or every 10-15 s from a speaker in front of the mouse in some trials.

Data analysis

Treadmill acceleration was calculated by digitally differentiating the treadmill velocity (which was sampled at 200 Hz).

Response probabilities for twitch and non-twitch trials were calculated by the proportion of trials with significant responses as long as there were at least 20 trials available for each trial type. A trial was classified as having an evoked response if, within the response peak period, the number of spikes exceeded four standard deviations of baseline firing (calculated from time-window of 100 msec preceding stimulus onset) during an equivalent duration of time. Response magnitudes were calculated for units where there were at least five trials with a significant evoked response in both twitch and non-twitch trials by averaging the number of spikes in those trials.

Results

Using the experimental approach described in Chapter 2, I observed that in addition to driving inferior colliculus (IC) activity, vermis photostimulation often evoked

a twitch-like movement of the mouse. These evoked movements were brief in duration, lasting < 300 msec, and relatively small in size, generating < 1° rotation of the treadmill.

I wondered whether this movement on the treadmill was akin to an acoustic startle reflex or reflective of a reflexive preparatory movement, and how the movement relates to IC neuronal firing. Startle and orienting behaviors, however, have been typically studied in the context of unrestrained, freely moving animals (Koch, 1999; Lauer et al., 2017; Yeomans & Frankland, 1996). To investigate how these stimulus-evoked behaviors manifest themselves when awake, moving mice are head-fixed on a treadmill, I measured the mouse motor response to auditory stimuli using a rotary optical encoder (ROE) (Figure 3.1A).

Auditory clicks can elicit startle responses and longer latency animal movements

Unexpected, sudden, loud (> 80 dB SPL) acoustic stimulation can elicit startle movements in vertebrate animals (Lauer et al., 2017; Yeomans & Frankland, 1996). These defensive behavioral responses are characterized by muscle contractions rostrocaudally along the body (Brown et al., 1991; Koch, 1999).

During a subset of experimental trials, mice were presented with loud auditory clicks of 100 dB SPL presented randomly (interstimulus interval of 10 to 15 sec) from a speaker directly in front of the animal. These loud clicks were able to reliably produce an acoustic startle response as characterized by a large motor response with an onset latency of 17 msec, as shown in an example mouse in Figure 3.1B. These short latency twitch movements made in response to suprathreshold startling sounds occurred at the same

onset latencies (10 – 20 msec in mice) as acoustic startle responses (ASRs) conventionally measured with a piezoelectric transducer (Koch, 1999). Thus, I was able to measure short latency twitch (startle) responses in mice using the treadmill ROE. Following the initial startle response, a longer latency movement occurred ~85 msec after the click.

When the same loud auditory clicks were presented at a fixed and predictable interval, the short latency component of the movement (startle response) was rapidly suppressed (Figure 3.1C). Instead, the mouse produced a movement with a latency of onset of 85 msec, which was longer than latencies associated with startle responses. Quieter auditory clicks presented at regular intervals also evoked delayed twitch movements at the same latency of onset but with reduced magnitude (Figure 3.1D). These findings suggest that predictable or quiet auditory stimuli can still produce a motor response, albeit one that that is more delayed than the startle twitch movement produced by loud, unpredictable sounds. These longer latency movements could represent reflexive preparatory movements produced by the mice in response to salient stimuli. Such preparatory movements can occur regardless of there being a preceding startle response, and may help the animal prepare for its next action (Novembre et al., 2018).

Vermis photostimulation elicits movement similar to auditory long-latency twitch movements

During the same experimental session in which I presented startling auditory stimuli, using the mouse as described in Figure 3.1, I photostimulated the cerebellar

vermis (lobules VI/VII) at a constant rate (1 Hz) and observed that the vermis evoked twitch resembles the movement made following loud auditory clicks (Figs. 3.2A, B).

The vermis evoked twitches are characterized by an identifiable early and late component which can vary across different animals (Figs 3.2C, D, E). The early component is characterized by a short latency movement that began 30 – 50 msec and attained peak velocity 50 – 60 msec after photostimulation onset (Figs. 3.2A, C, D). The timing of the early component of vermis-evoked movement resembled the acoustic startle response in both its fast onset and short duration (Figs 3.2A, B).

The late component of animal movement following vermis photostimulation typically has an acceleration onset latency of 70 – 100 msec, with velocity peaking around 120 – 140 msec following light onset (Figs 3.2A-E). This long latency movement resembled the reflexive preparatory movement following auditory stimuli, especially ones that are predictable or quieter. Similarly, both delayed latency movements were typically larger and longer lasting than the startle response.

Different mice had different movement patterns in response to vermis photostimulation (Figs 3.2C, D, E). This individual variability was similar to the differences described in the acoustic startle response (Koch, 1999). Some mice consistently produced a startle movement following vermis stimulation, while the startle response was suppressed in other mice. The mouse in Figure 3.2E, for example, had a completely suppressed startle to vermis photostimulation, but still made a long latency preparatory movement.

The startle twitch could also vary in its direction in different mice, as measured by treadmill movement. For example, the startle produced by the mouse in Figure 2.2C manifested as a brief backwards movement (as characterized by the negative treadmill velocity) that peaks at 55 msec, followed by a brief forwards movement (as measured by the positive treadmill velocity), and finally a preparatory response. In contrast, the treadmill only moved backwards when the mouse in Figure 2.2D startled. Despite this variability in movement pattern, the timing of the startle relative to vermis stimulation was consistent. When it occurred, the startle movement had a peak velocity around 55 msec, while the preparatory movement peaked around 130 msec. All mice produced a longer latency preparatory response following vermis photostimulation, as might be expected from stimulation of the oculomotor vermis, which is known to be involved in saccadic eye movements and head movements (Dugué, Tihy, Gourévitch, & Léna, 2017; Guerrasio, Quinet, Büttner, & Goffart, 2010; Kurkin et al., 2014; Sun, Junker, Dicke, & Thier, 2016). These results demonstrate that vermis photostimulation could evoke characteristic movements resembling those produced by auditory clicks. Mice exhibited individual differences in the movement patterns of their respective twitch responses, but the timing of the startle and preparatory components of the movement was consistent.

Early evoked IC firing co-occurs with, but is dissociable from, early twitch movement

In order to assess whether and how animal movement was related to IC neural activity, I performed *in vivo* extracellular recordings in awake mice while photostimulating the vermis, and compared the firing responses of IC units with the motor responses. As described in Chapter 2, vermis photostimulation elicits several types

of responses in IC neurons. Neurons that were excited by vermis photostimulation exhibited an early latency response, a long latency response, or both early and late firing responses.

In neurons with short-latency firing responses, the early peak in firing occurs at around the time of the startle movement but could be disassociated from it. Figure 3.3A depicts the average firing rate of a single IC unit with an early peak response (black) and the average treadmill acceleration following vermis photostimulation (red). This unit had an early firing response latency of 40 msec, which preceded the early acceleration peak at 50 msec (Fig 3.3A). By contrast, the early firing for the unit in Fig 3.3C was at 56 msec, slightly following the early peak in acceleration at 49 msec. Notably, early firing was still evoked in the absence of a short latency movement, when mice had a suppressed startle. For example, the animal in Figure 3.3B did not produce an early motor response during any of the photostimulation trials, but the first peak of the recorded IC firing was evoked at a consistent onset latency of 43 msec, peaking at 52 msec. The mouse did produce a late movement, which reached peak acceleration at 105 msec.

As a population, IC neurons with early evoked responses to vermis photostimulation typically fired before or during short-latency startle-like movements, when these movements occurred (Fig 3.3D). When the animal did not produce an early movement, early evoked responses still occurred with the same onset latency (30 – 60 msec), and preceded the long latency movement by > 40 msec (Fig 3.3D). Given the long duration between the early neuronal response and late movement, it is unlikely that the evoked firing was a motor-related corollary discharge. In addition, these results

indicate that the vermis-evoked early response was not causally linked to the early movement.

Early vermis evoked IC firing correlates with late twitch movement

In Figures 3.4 A – C, I sorted the photostimulation trials of single IC units by the absolute treadmill movement magnitude (within a time window of 0 to 300 msec from photostimulation onset). The rasters of three IC neurons' spiking activity are overlaid atop a heatmap of the treadmill velocity during their respective photostimulation trials (Figs 3.4 A – C). Blue on the heatmap denotes a negative velocity where the treadmill moves backwards, while red shows positive velocity and forward movement.

Not every vermis photostimulation presentation evoked a motor response, which allowed for the comparison of vermis driven IC firing relative to animal motor activity. I noticed that some IC neurons with early response had increased evoked activity in photostimulation trials with animal twitch (Fig 3.4C). When the average firing rates for the cell in Figure 3.4C were calculated in twitch versus non-twitch trials, for example, the early evoked response was smaller when the treadmill did not move (64 Hz max evoked firing) than when it did (180 Hz max evoked firing) (Fig 3.4D). The decreased response reflected a higher probability of early evoked firing in twitch trials (94.5%) than in non-twitch ones (52.6%). A trial was defined as having an evoked response if, within the late peak response time window, there were more spikes than 4 standard deviations above the baseline number of spikes for the same time period (calculated from the 100 msec time window preceding stimulus onset).

Early vermis evoked firing typically occurred with higher probability in trials with animal twitch (49.0 ± 7.7%) compared to trials without (26.6 ± 5.6%) (Fig. 3.4E, left). However, in trials with an evoked response, the number of elicited action potentials remained the same regardless of animal movement, showing that the late movement does not modulate response strength (Fig 3.4E, right).

These findings together show that the early IC neural response produced by vermis stimulation were invariant to when the twitch occurs, and typically preceded or co-occurred with the movement, indicating that the twitch was not causing the early evoked response. The early evoked firing occurred at higher probabilities when the animal twitched, but did not increase in response strength. Because the animal twitch was largely composed of the late accelerative component and early evoked firing did not depend on the early accelerative component, these results indicate that there is a correlation between whatever drives the early evoked response and the late acceleration.

Long latency vermis-evoked responses co-occur with late treadmill acceleration

Because the long latency neural responses occurred after any startle response and around the time of the late treadmill acceleration (preparatory movement), I analyzed whether these cells were affected by the later presumed preparatory movement. Late evoked responses occurred at around the same time as the late treadmill acceleration, on average 7 ± 2 msec before the peak of late acceleration (Fig 3.5E).

I observed that the second evoked peak typically did not occur in trials without an evoked twitch movement. Figures 3.5A shows the spiking activity of an IC neuron with

two peaks of firing following vermis photostimulation sorted by treadmill velocity. The late evoked peak in firing occurred only in trials with treadmill movement, while the early firing occurs even in trials without movement (Fig 3.5A). I separated out the photostimulation trials without twitch (Fig 3.5B, left) and with twitch (Fig 2.3B, right), and compared the average firing rate of the IC neuron (black line) and average animal acceleration (red line) in each type of trial. In the non-twitch trials, the cell only had an early response peak, which occurred with a latency of 32 msec from photostimulation onset with a maximum evoked firing rate of 150 Hz (Fig 3.5B, left). As expected for trials without animal twitch, there was no change in treadmill acceleration. In twitch trials, the cell also had a second peak of activity, which occurred 90 msec after light pulse onset with an evoked peak of 93 Hz above baseline (Fig 3.5B, right). In addition, the early response peak has a higher probability of firing than in trials without twitch.

In IC neurons with only long latency responses, the evoked response is also correlated with animal twitch. The example neuron in Figures 3.5C and D only responded to photostimulation in trials where the animal also produced a twitch. The evoked response had a peak of 99 Hz and occurred with an onset latency of 98 msec, which is after the animal startled at 64 msec. In the 49/140 (35%) photostimulation trials with did not produce a twitch, there was no evoked neural activity.

Across 12 single IC units with long latency responses to vermis stimulation in which I measured > 20 twitch and non-twitch trials, photostimulation trials with animal twitch had a higher probability of a late evoked firing response (49.3 ± 7.4%) compared with trials without a twitch (16.0 ± 5%) (Fig. 3.5F, left), though the response magnitude did not change (Fig 3.5F, right).

In a subset of recordings, I was able to reconstruct the approximate location of the recorded neurons. When mapped, the majority of neurons (5/7 late response neurons) with twitch-correlated responses were found in the external cortex of the IC (ECIC). By contrast, only one each were found in the central nucleus and the dorsal cortex.

In addition, the majority of long latency evoked responses (18/23 single and multiunits) occurred slightly before the second peak of treadmill acceleration (Fig 3.5E). These results indicate the IC long latency response to vermis stimulation was correlated with the animal movement and may correspond to a motor corollary discharge signal.

Auditory evoked movement can also evoke IC responses

Given that IC neural activity is correlated with animal movement, I analyzed whether the long latency movements sometimes produced by auditory clicks could elicit similar IC activity preceding the movement. Figure 3.6 shows two example neurons with sufficient trials that produced an animal twitch response following a quieter click stimulus. The neuron in Figure 3.6A and B had a short latency response to the click as well as a later response that was correlated with the presence of an animal twitch. Notably, the second peak of IC activity occurred 31 msec after the click, which was before peak twitch acceleration at 105 msec, indicating that the movement itself was not what drives the neural response (Fig 3.6A, B). This second peak of activity also occurred in trials without movement, albeit at a reduced frequency.

A second example neuron is shown in Figure 3.6C and D. This cell had a strong, fast response to the auditory click, followed by a brief suppression. In the trials with

animal movement, there was a second excitatory response with a latency of 90 msec around the time of the twitch (100 msec). This long latency response only occurred in trials when the animal twitched following a relatively quiet (70 dB SPL) auditory click.

Interestingly, both of these neurons with evoked responses preceding click-evoked animal twitch also had vermis evoked responses that were modulated by animal twitch. The neuron in Figure 3.6A had increased probability of early and late firing following animal twitch in vermis photostimulation trials (Fig 3.4C, D). Animal twitches also increased vermis evoked late firing probability in the cell depicted in Figure 3.6C (Fig. 3.5C, D). These results suggest that evoked responses in some IC neurons are correlated with animal twitch, whether it follows vermis photostimulation or an auditory stimulus. It is worth noting that I did not realize auditory clicks could evoke movement-related activity in the IC until I analyzed the data, so these experiments were not designed to assess this relationship. Experiments explicitly testing how real behavioral signals affect IC firing should be conducted in the future.

Discussion

Given the IC's known acousticomotor functions, how do movement and neuronal responses in the IC relate? In this study, I showed that evoked neuronal activity in the IC can be either related or unrelated to movement. Both vermis photostimulation and acoustic clicks could produce movements that had either an early and late component, or solely a late component, and motor responses to vermis stimulation were similar to those elicited by auditory stimuli. A parsimonious explanation for the similarities in these

motor responses is that early movement corresponds to a startle response while late movement corresponds with a postural movement that enables the mouse to prepare for a subsequent action.

The first peak of vermis evoked IC activity is dissociable from the startle movement; it occurred in mice that did not startle, indicating that the two are not causally linked. However, probability of early firing correlated with the occurrence of the late component of movement, although the magnitude of response was the same. The later evoked firing, by contrast, was predominantly time-locked to the later movement, and thus is consistent with a motor corollary discharge signal. In addition, a small subset of auditory click trials produced animal movement, which also correlated with evoked IC firing at latencies that corresponded to vermis-evoked alerting or motor corollary discharge signals. This study demonstrates that IC alerting responses to different types of salient stimuli are not causally linked to movement, while motor corollary signals are correlated with movement.

Animals with suppressed startle response still produce movement to stimuli

Strong, unexpected stimuli of different modalities can generate startle responses in animals, and probably helps protect the animal from potential harm (Yeomans et al., 2002). It is also possible that a startle movement interrupts ongoing behavior, thereby allowing the animal to prepare for action (Koch, 1999). However, startling is associated with whole-body muscle contraction and eye closure, which could result in transient decreases in motor coordination, attention, and visual processing (Yeomans et al., 2002).

In light of these considerations, the long latency twitch movement I observed in response to acoustic and vermis stimulation (Figs 3.1, 3.2) could assist in recovery from a startle and prepare the animal for subsequent behavioral responses.

In my experiments, mice head-fixed on a treadmill also generated a similarly fast startle measurable by a ROE in response to loud, unpredictable auditory clicks (Fig 3.1B). The short latency startle was also often accompanied with a longer latency movement (Fig 3.1B). In addition, mice did not always startle, even to loud sounds, but could still produce a measurable motor response at a longer latency (Fig 3.1C). Vermis photostimulation evokes an animal twitch with an early startle and later movement (Fig 3.2). Similar to auditory click experiments, some mice had a suppressed startle movement and only produced a long latency twitch (Fig. 2.2E, 2.4C, 2.6E). These results suggest that the animals detect and respond to the stimuli even when they are not startled by them. In humans, salient, but not startling, stimuli produce stereotyped motor output that is coupled with a cortical saliency signal (Novembre et al., 2018). Here, the late movement probably functions as a preparatory movement that aids the animal in resetting post-startle and making an appropriate behavioral response to stimuli (e.g.: to freeze, escape, or orient).

Vermis stimulation can produce startle and orienting-related movement

Brief, cerebellar vermis photostimulation evoked an animal twitch with early startle and late preparatory components (Fig 2.2). The cerebellar vermis is involved with motor coordination and postural control (Mathy & Clark, 2013; Nisimaru, 2004; Schmahmann, 2004). The fastigial nucleus (FN) makes dense projects to motor thalamic

subnuclei, including VM, MD, and VL (Batton, Jayaraman, Ruggiero, & Carpenter, 1977; Katoh et al., 2000). Transsynaptic rabies tracing reveals that primary motor cortex (M1) and premotor areas representing both proximal and distal body parts provide major inputs to lobules V-VIII of the vermis (Coffman et al., 2011). Thus, there are reciprocal circuits between motor cortices and the cerebellar vermis. The vermis may play a key role in evoking postural adjustments made in preparation for movement (Coffman et al., 2011; Cordo & Nashner, 1982). Indeed, vermal lesions disrupt the plasticity of anticipatory postural adjustments (Moberget et al., 2016).

In my vermis photostimulation experiments, the optic fiber was targeted to lobules VI/VII, which is also known as the oculomotor vermis, a structure that controls targeted saccades (Voogd & Barmack, 2005). Electrical stimulation of the oculomotor vermis and downstream neurons in the caudal fastigial nucleus (FN) has been shown to elicit saccades in primates (Noda & Fujikado, 1987). Given the orienting function of saccadic eye movements, it seems probable that the postural adjustments produced by photostimulation of the oculomotor vermis are made in anticipation of orienting behavior.

The vermis evoked startle response could be mediated by known projections from the FN to the brainstem nuclei involved with startle production, the caudal pontine reticular (PnC) and gigantocellular neurons (Gi) (Andrezik, Dormer, Foreman, & Person, 1984). The FN also innervates the vestibular nuclei (Batton et al., 1977), which could contribute to a vestibular-evoked startle response (Yeomans et al., 2002).

There are several caveats and considerations for interpreting the treadmill movement data. Vermis evoked movement could be due to changes in animal balance on the treadmill. However, because the mice in these experiments were head-fixed,

vestibular sensors of rotation and acceleration in the inner ear were not activated. In addition, the treadmill ROE did not capture other movements that the animal may be making during the experiment. For example, loud auditory stimuli could produce pinna movements, which would not be directly measured in my data, and could produce a somatosensory signal that gets relayed to the IC. However, from my observation of the mice during the experiments, vermis photostimulation did not elicit noticeable pinna movement even when the mouse produced a full body twitch, though loud acoustic clicks sometimes did. The treadmill movement caused by auditory and vermis stimulation was similar (Fig 3.2)

Functional role of cerebellar vermis and fastigial nucleus in arousal

The FN has been implicated in the regulation of arousal. Activation of the FN has been shown to induce pupil dilation (Fadiga, Manzoni, Sapienza, & Urbano, 1968; Snider & Maiti, 1976), aggressive behaviors (Dietrichs, 1984; Snider & Maiti, 1976), and changes cardiovascular responses (Lisander & Martner, 1973, 1975; Miura & Takayama, 1988; Nisimaru, 2004; Nisimaru & Kawaguchi, 1984; Snider & Maiti, 1976). The FN densely innervates brain areas corresponding to the reticular activating system (RAS), and, in a classic 1949 paper, FN stimulation was described to activate the RAS (Moruzzi & Magoun, 1949). Seminal research conducted by Mircea Steriade then showed that FN stimulation generates cortical desynchronization via the reticular activation system (RAS) in addition to motor outputs (Steriade, 1995, 1996).

The photostimulation procedure used in my experiments could be producing an alerting signal by effectively silencing ongoing Purkinje cell activity, either directly (L7-cre; ArchT) or indirectly via activation of inhibitory interneurons (NOS1-ChR2). This artificially induced synchronized pause of PC firing would disinhibit fastigial nucleus neurons (Person & Raman, 2012; Ramirez & Stell, 2016), and may mimic teaching signals conveyed from the inferior olive via climbing fibers. Inferior olive neurons are activated by unexpected events and movement errors, and elicit complex spikes in PCs followed by a synchronized pause of PC simple spike firing. Thus, brief optogenetic photostimulation of the vermis could generate an alerting signal that is broadcast from the cerebellum to the rest of the brain, including to the IC.

Early IC evoked firing consistent with alerting signal from vermis

Vermis photostimulation evoked an early peak response in IC occurring at a similar time to vermis-elicited startle movement – how do the two relate? One possibility is that the early evoked response was driven by the animal startle movement. However, the first peak in IC firing was present even when an early startle movement did not occur (Fig 3.3, 3.4C). In addition, because the early firing occurred around the time of movement or well before its onset (Figs 3.3), the early evoked response was not a result of somatosensory reafference which would presumably occur after movement. The early peak firing was also not a response to sounds that may be produced by the twitch because the early evoked firing persisted even when mice were ear plugged (see Results Chapter 4).

The early evoked response probability of the IC correlated with the occurrence of long-latency preparatory movements made by the mouse (Figs 3.4C, 3,4E). Because corticofugal neurons in the auditory cortex projecting to IC mediate innate, sound-induced escape behavior in mice (Xiong et al., 2015), one possible explanation for the correlation between early evoked response and late movement is that the early evoked response drives production of preparatory movement. However, the early evoked response occurred 40 – 120 msec prior to the onset of the late movement (Fig 3.4E), which is a relatively long interval of time. In addition, the early evoked activity could also occur in the absence of late movement, indicating that the two are dissociable (Figs 3.3A).

The timing of the early firing peak in IC, which occurred 30 – 60 msec after vermis photostimulation, corresponds with the timing of N1 auditory cortical salience signal, a correlate of auditory feature- and event-detection (Horváth, 2015). In humans, this event-related potential (ERP) is characterized by a negative peak of activity measured by scalp electrodes occurring 100 msec after a salient auditory stimulus presentation (Horváth, 2015; Novembre et al., 2018). The N1, also known as N100 for its response latency, has been described as a stable measure of sensory gating deficits in neuropsychiatric disorders such as schizophrenia (Maxwell et al., 2004). In mice, a negative peak around 40 msec bears resemblance to the human N100 or N1, though there is debate about the analogous relationship between auditory evoked potentials in humans and in rodents (Maxwell et al., 2004; Siegel et al., 2003). The N1 response is mediated by the auditory cortex, and its correspondence with the latency of the IC early evoked response suggests that vermis modulation of the IC occurs via thalamocortical circuits.

In addition, a recent study in humans found that unexpected, substartle stimuli of different modalities produced a tightly coupled motor response alongside cortical modulation, suggesting that detecting salient stimuli is tied to preparation for action (Novembre et al., 2018).

IC activity correlates with pupil dilation, which is a measure of arousal (Joshi, Li, Kalwani, & Gold, 2016), as well as behavioral context, like reward size in a trained auditory task (Metzger et al., 2006). In addition, the majority of neurons in the IC decrease their responses to repetitive sounds, but not to infrequent ones, a characteristic known as stimulus-specific adaptation (SSA) (Ayala, Pérez-González, Duque, Nelken, & Malmierca, 2012; Ayala, Pérez-González, & Malmierca, 2016; Ayala & Malmierca, 2015; Duque & Malmierca, 2015; Malmierca et al., 2009; Shen, Zhao, & Hong, 2015; Zhao, Liu, Shen, Feng, & Hong, 2011). Neurons with SSA were found distributed throughout the IC, including the CIC, though neurons with higher levels of SSA ("novelty" neurons) were more prevalent in lateral and dorsal IC (Ayala & Malmierca, 2015; Malmierca et al., 2009).

From these converging lines of evidence, a parsimonious explanation is that early peak activity in the IC represents an alerting signal produced by the vermis to a salient stimulus event. The more salient the event, the more likely an animal is to make an anticipatory preparatory movement, with startle responses occurring at higher thresholds. Alerting signals can be evoked for events that do not rise to the level of triggering preparatory motor output, but are more likely for (and thus correlated with) more salient events that do produce a movement.

Late evoked firing may represent an efference copy of anticipatory postural movement

The late IC evoked response typically depended on the late component of movement, and did not occur with high probability in absence of the late acceleration (Figs 2.4, 2.6). The late peak occurred around the time of the late acceleration, suggesting that the late evoked IC activity reflects either a corollary motor discharge or a response to the sensory reafference of the late movement (Fig 3.65E).

Connections related to both types of signals have been identified previously in the IC. The IC, particularly ECIC and the CIC, is reported to receive projections from ipsilateral motor cortex in cats (Cooper & Young, 1976). In addition, oculomotor saccade signals have been measured in the majority of ECIC neurons in the monkey (Bulkin & Groh, 2012; Gruters & Groh, 2012a; Porter et al., 2007). The ECIC also receives inputs from somatosensory cortex and the dorsal columns, which could convey sensory information about the animal's movement (Aitkin et al., 1981; Aitkin & Zimmermann, 1978; Jain & Shore, 2006; Lesicko et al., 2016; Zhou & Shore, 2006). The CIC may also receive somatosensory feedback via input from the dorsal cochlear nucleus, which has tactile responses related to the head (Kanold & Young, 2001).

However, the majority of late responses I recorded occurred prior to the peak of the late acceleration, which would not be consistent responses from sensory feedback of the preparatory movement (Figs 3.5E). The late peak response was not well correlated with early startle movement, and occurred when the animal only made a preparatory movement (Fig 3.5F). Because the firing was time-locked with the late movement driven by vermis stimulation, the timing of the late peak was not consistent with a sensory re-afference made in response to the animal movement. I therefore hypothesize

that the late firing peak corresponds to a motor corollary discharge signal in the IC for the whole-body preparatory movement.

Functional implications of early and late evoked firing in different anatomical subdivisions of IC

Although neurons with early and late peak responses could be found throughout the IC, their predominant locations and connectivity could potentially underlie different functions. The IC is required for prepulse inhibition (PPI), which is the suppression of the acoustic startle response (ASR) by the presentation of a weak stimulus preceding a startling one (Braff, Geyer, & Swerdlow, 2001; Brown et al., 1991; Lauer et al., 2017; Yeomans & Frankland, 1996). The ECIC projects to areas that could modify startle behavior, such as the pons and the midbrain cholinergic centers in the pedunculopontine tegmentum (PPTg) and laterodorsal tegmentum (LDTg) (Huffman & Henson, 1990; Lauer et al., 2017). These cholinergic nuclei have been implicated in mediating prepulse suppression of the acoustic startle reflex (Fendt et al., 2001; Jones & Shannon, 2004; Lauer et al., 2017; Maclaren, Markovic, & Clark, 2014; Yeomans et al., 2006). The alerting signals in the ECIC could carry information about salient, below startle-threshold stimuli to reduce caudal pontine reticular output to spinal cord motoneurons, thereby reducing startle response amplitude. Non-GABAergic neurons in auditory, extramodular regions of the ECIC project to the superior colliculus (Lesicko & Llano, 2019), and is positioned to provide motor corollary discharge signals about preparatory movement for conscious orienting behavior. Notably, IC neurons with two peaks of evoked firing following vermis photostimulation were found predominately in ECIC (Fig 2.6).

The dorsal cortex (DCIC) projects to the central nucleus of the inferior colliculus (CIC) as well as the contralateral IC, and receives extensive top-down input from the auditory cortices (Games & Winer, 1988; Winer, Larue, Diehl, & Hefti, 1998). Early and late evoked responses in the DCIC could be modifying auditory information processing. The early alerting response in DCIC could help modulate auditory signals in the CIC, while the late corollary discharge signal could contribute to the anticipation of orienting towards or away from an auditory stimulus, or in the suppression of self-generated sound.

The subdivisions of the IC are highly interconnected, which could explain the heterogeneity of responses described in this chapter (Coleman & Clerici, 1987; Winer & Schreiner, 2005). The ECIC projects to both the DCIC and CIC, which could be account for the presence of late vermis evoked firing responses observed in those regions. The intracollicular projections from ECIC originate from extramodular regions and are mostly non-GABAergic (Lesicko & Llano, 2019). In a pilot study, I inactivated ipsilateral auditory cortex using optogenetic and pharmacological approaches to but did not find changes vermis evoked responses in the IC as would be expected from a polysynaptic thalamocortical circuit connecting vermis to IC. Intracollicular signaling connecting both hemispheres of the IC might have compensated for unilateral inactivation of top-down auditory cortical input. A bilateral approach to inactivating the auditory cortices may be able to block vermis evoked activity in the IC.

Figures

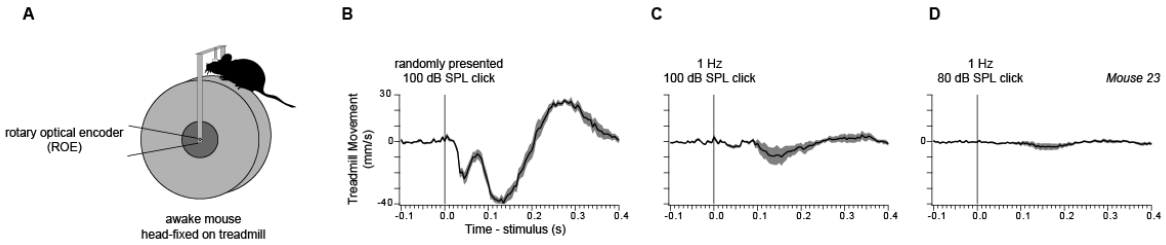


Figure 3.1: Auditory clicks produce measurable animal movements on the treadmill.

A. Schematic of treadmill set-up with built-in rotary optical encoder (ROE) for movement measurements.

B. Representative average trace of animal movement following 100 dB SPL auditory click presented randomly (10 – 15 s interstimulus interval). A negative velocity means the animal moved backwards on the treadmill, while a positive velocity means it moved forwards.

C, D. Movements of same animal following 100 and 80 dB SPL click, respectively, presented every 1 s. Traces are presented as mean ± sem.

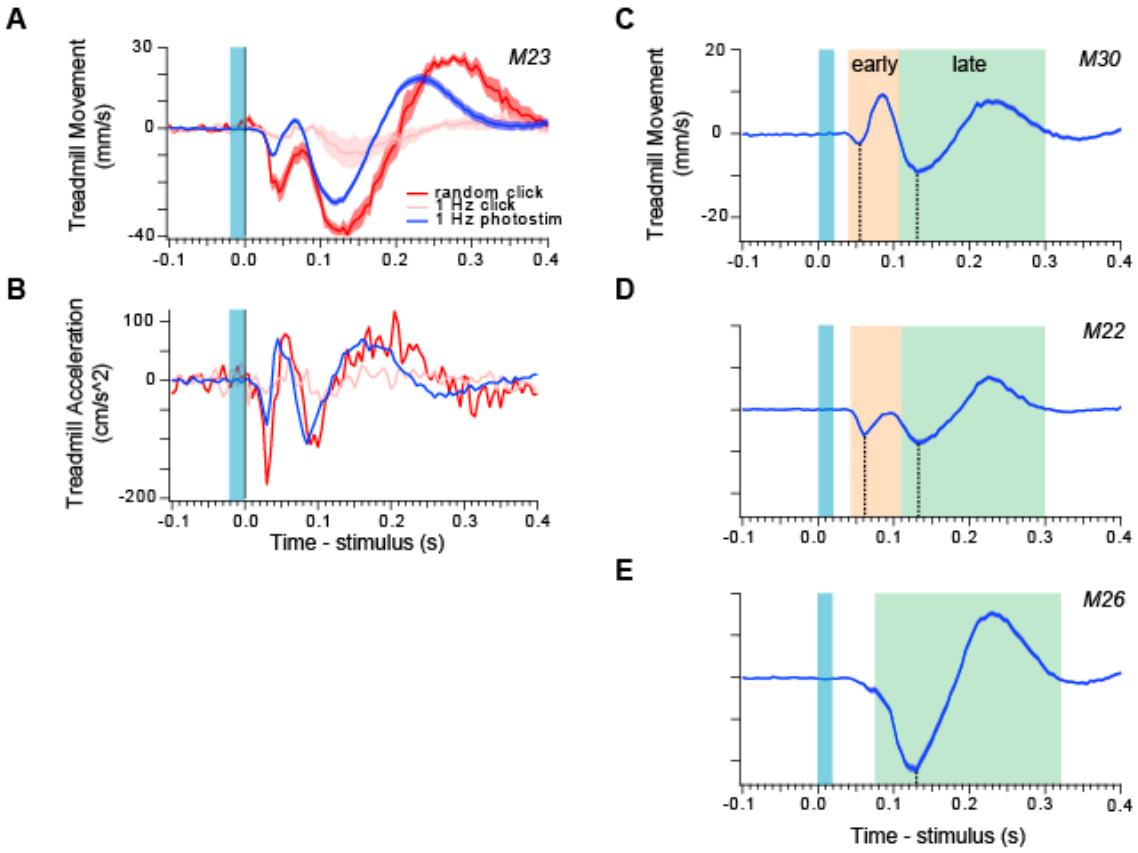


Figure 3.2: Vermis photostimulation produces a two-component movement that resembles acoustically driven movements

A, B. Comparison of average animal movement following loud auditory clicks (same as in Figure 3.1) and vermis photostimulation. Click onset is aligned to photostimulation offset. (A) shows treadmill velocity and (B) depicts its acceleration.

C, D, E. Average movement following brief vermis photostimulation in different mice.

C, D. Vermis evoked twitches with both early (yellow) and late (green) movement components.

E, Vermis evoked twitch with only a late component.

Dashed lines denote latency of peak velocity of the early and late components. Treadmill velocity traces shows mean ± sem.

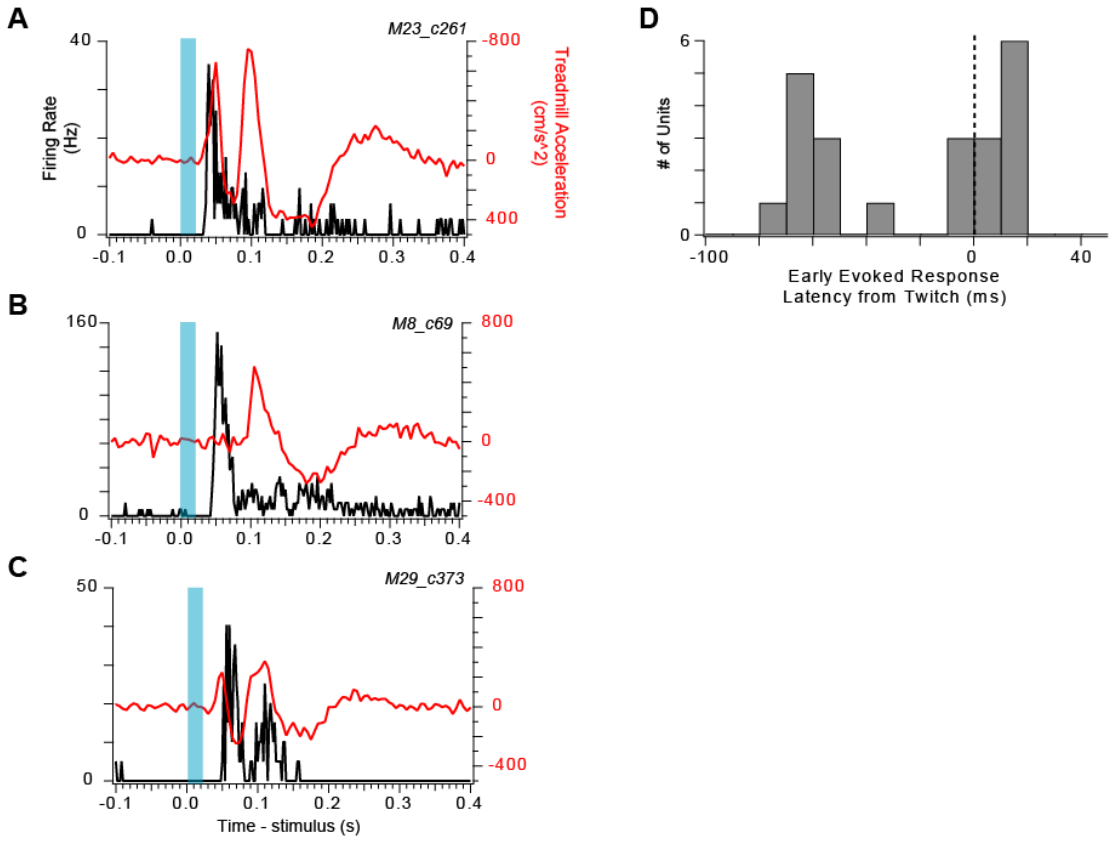


Figure 3.3: Early evoked firing co-occurs with but dissociable from early startle movements

A-C. Representative IC neurons with early evoked response to vermis photostimulation (black) and average treadmill acceleration (red).

D. Histogram of the onset latency of early firing relative to peak acceleration of treadmill movement (n = 22 IC neurons). Note that the early response onset latencies remain consistent (30 – 60 msec following vermis photostimulation), but the latency of twitch movement is not consistent because some mice did not produce an early startle movement. In these cases, the early evoked response preceded the late movement by >40 msec.

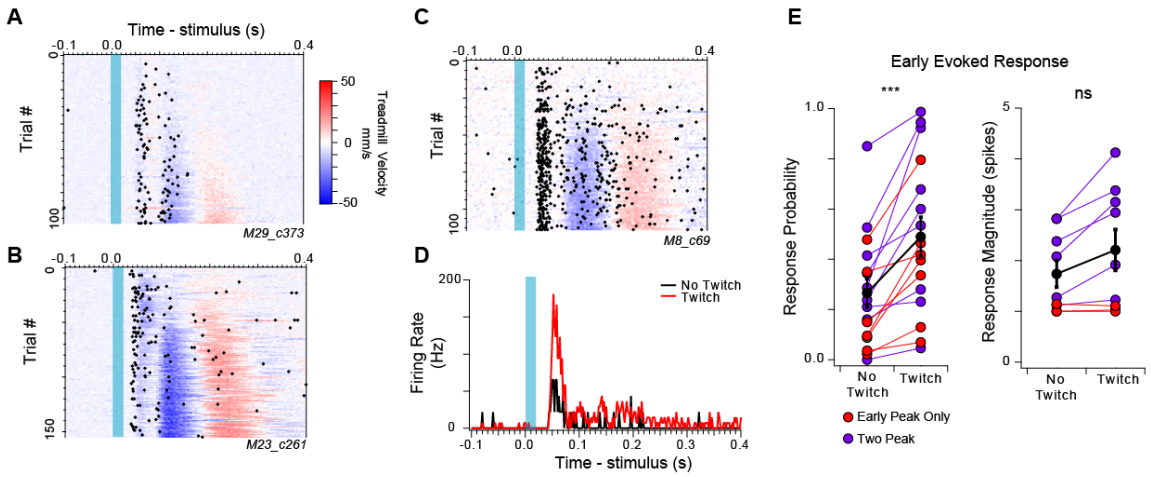


Figure 3.4: Increased early firing probability in late movement vermis photostimulation trials

A. Raster of representative IC neuron with two peaks of evoked activity during vermis photostimulation trials sorted by treadmill movement. Same single unit as in Figure 3.3C.
B. Example IC neuron with only early evoked activity sorted by treadmill movement. Same single unit as in Figure 3.3A.
C. Same convention as in A and B. Same single unit as in Figure 3.3B.
D. Vermis evoked activity of neuron in (C) in non-twitch and twitch trials.
E. Left, early firing response probability during non-twitch and twitch trials (n = 12 single units). Right, early response magnitude in non-twitch and twitch trials with significant evoked responses (n = 6 single units).

***P<0.001. ns, not significant. Mean ± sem.

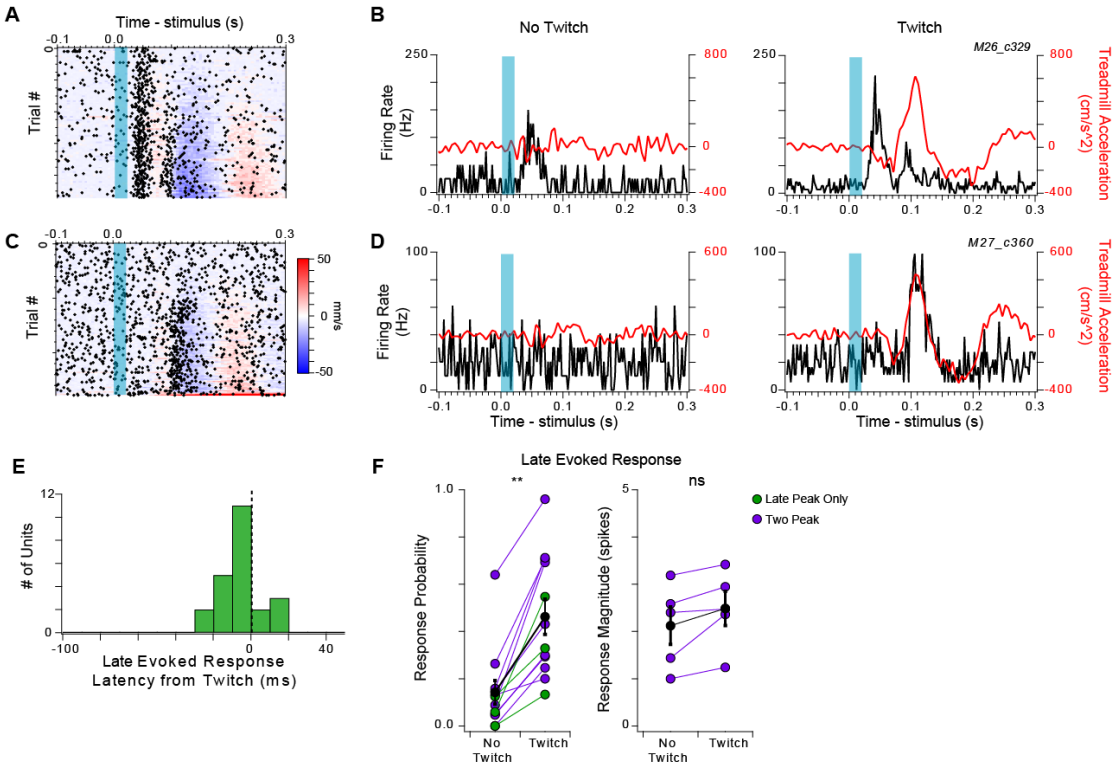


Figure 3.5: Long latency vermis evoked response in IC correlates with late animal movement

A. Raster of representative IC neuron with two peaks of evoked activity during vermis photostimulation trials sorted and overlaid by treadmill movement.
B. Same neuron as (A) with average peristimulus time histogram average of evoked activity (black) and the average treadmill acceleration (red) during trials without animal twitch (left) or with animal twitch (right).
C. Representative IC neuron with only long latency evoked response to vermis stimulation. Same conventions as (A).
D. Average evoked activity and treadmill acceleration during twitch and non-twitch trials of neuron in (C).
E. Histogram of onset latencies of late response peaks relative to onset of late treadmill acceleration (n = 23 IC neurons with late evoked response)
F. Left, late firing response probability during non-twitch and twitch photostimulation trials (n = 16 single units). Right, response magnitude in non-twitch and twitch trials with significant evoked firing (n = 5 single units).

**P < 0.01; ns, not significant. Mean ± sem.

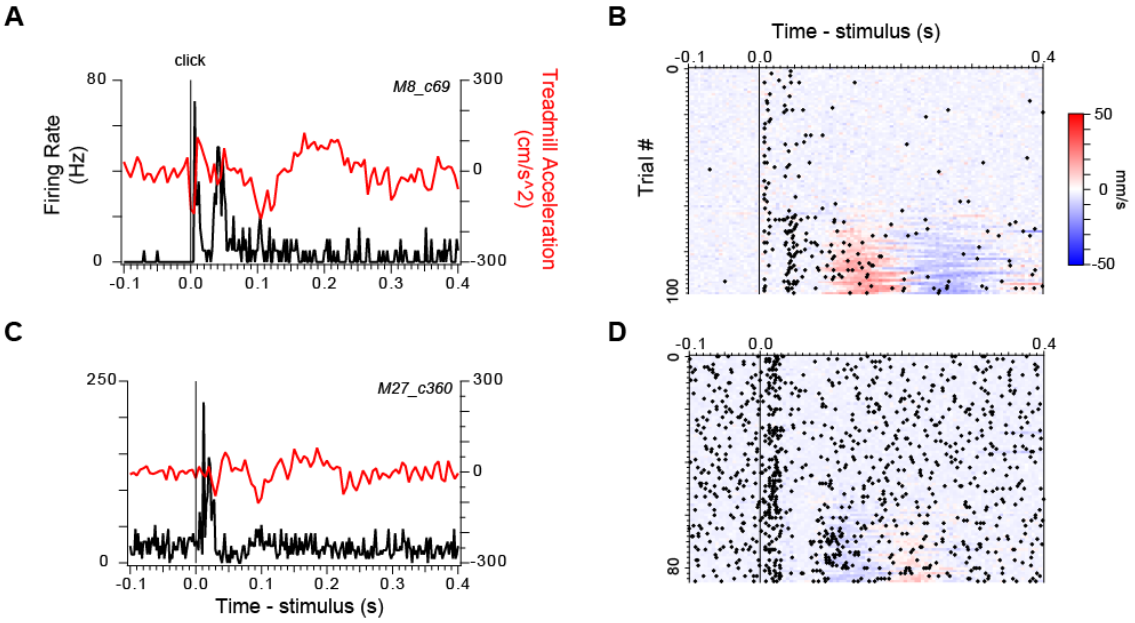


Figure 3.6: Long latency movements evoked by auditory clicks also evoked IC responses

A. Example neuron with two peaks of activity (black) following auditory click (87 dB SPL) and the average treadmill acceleration (red).
B. Same neuron in (A) with each click trial sorted by animal movement.
C. Neuron from Figure 2.4C and D with twitch following auditory click (70 dB SPL) and average treadmill acceleration.
D. Same neuron as in (C) with each click trial sorted by movement.

Chapter 4. Animal running gates inferior colliculus response to cerebellar photostimulation and auditory clicks

Introduction

Behavioral state changes the functional demands of the brain, especially in circuits involved with sensory processing and defensive behaviors. In the visual system, locomotion increases responsivity to visual stimuli. In the primary visual cortex (V1), responses to visual stimuli increased by more than two-fold during animal running compared with rest, without significant changes in tuning (Dadarlat & Stryker, 2017; Niell & Stryker, 2010). Almost half of V1 neurons are driven by combinations of visual and running speed in mice, even in the dark (McGinty et al., 2013).

By contrast, the auditory system has suppressed responses during animal locomotion. Responses to auditory tones and stimulation of projections from the auditory thalamus are reduced in auditory cortical excitatory neurons during animal running (Nelson & Mooney, 2016; Nelson et al., 2013; Schneider et al., 2014; Zhou, Liang, et al., 2014). Locomotion also modulates activity at the level of the medial geniculate body (auditory thalamus) where evoked responses to sounds are reduced (Williamson et al., 2015). In addition, both the dorsal cochlear nucleus and auditory cortex have reduced responses to self-generated sounds (Rummell et al., 2016; Singla et al., 2017). However, despite the preponderance of evidence showing behavioral state effects on the auditory system, to the best of my knowledge, no studies have been conducted on the effect of animal locomotion on inferior colliculus (IC) function.

The IC is an essential midbrain hub for ascending and descending auditory inputs (Kudo & Niimi, 1980; Winer, Chernock, Larue, & Cheung, 2002; Winer & Schreiner, 2005). The IC also plays important roles in defensive behaviors, including producing running behaviors (Xiong et al., 2015). Graded excitation of the IC through electrical stimulation generates arousal, freezing, and flight behaviors (Brandão et al., 1993, 1988). Sound-induced escape behavior is dependent upon neural activity in the IC shell region, which projects to the dorsal-lateral periaqueductal grey, a midbrain defense structure (Xiong et al., 2015). Animal running state is therefore likely relevant to IC function.

The results presented in previous chapters of this dissertation show that the IC receives robust cerebellar vermis input. Animal locomotion also modulates cerebellar cortex activity (Ghosh et al., 2011; Hoogland, Gruijl, Witter, Canto, & Zeeuw, 2015; Ozden, Dombeck, Hoogland, Tank, & Wang, 2012; Powell, Mathy, Duguid, & Michael, 2015; Sauerbrei, Lubenov, & Siapas, 2015). Recently, running was found to improve cerebellum-dependent associative learning of delay eyelid conditioning (Albergaria, Silva, Pritchett, & Carey, 2018). Does locomotion affect vermis input to the IC?

In this study, I address whether and how running modulates IC evoked responses to vermis optogenetic stimulation and auditory clicks. My experiments reveal that locomotion is associated with decreased responsivity in IC to both cerebellar and auditory inputs, as well as a general increase in spontaneous activity. These results suggest that running gates off or reduces the efficacy of auditory and cerebellar input to the IC, which elucidates the effects of behavioral state on a previously understudied, but essential, auditory and defensive structure.

Methods and Materials

Animal surgeries, optogenetic stimulation, and extracellular recording experiments were conducted as previously described in Chapters 2 and 3.

Recordings and optogenetic stimulation in ear plugged mice

In experiments with ear plugged mice, 3 NOS1-ChR2 mice (2 male and 1 female) were anesthetized with isoflurane (1-2% by volume). Both ears were plugged with a small piece of earplug foam hand-cut to fit into the murine ear canal. After visually inspecting that the ear plugs were snug in the ear canal, the outer ear and ear plug were covered with Kwik-cast (World Precision Instruments) to prevent the plugs from being removed.

Auditory clicks were presented during recording sessions to ensure that the ear plugs were attenuating sounds. Mice were less responsive to loud clicks as measured by local field potential recordings (low-pass filter 1 – 300 Hz; 2k gain) and evoked movements.

The sound produced by mouse and treadmill movement were measured with a Sokolich microphone (Sokolich).

Data analysis

Trials were categorized as running if the treadmill velocity in the 300 msec time window before stimulus onset exceeded 10 mm/s. Running trial firing rates were averaged if there were 20 or more trials for both stationary and running trials

Results

Animal movement modulates IC response to cerebellar photostimulation and baseline activity

During vermis photostimulation trials, mice would sometimes spontaneously run on the treadmill for prolonged bouts that lasted for several seconds. For the analyses of IC firing to auditory clicks and vermis photostimulation in Chapters 2 and 3, I excluded these trials because I noticed that IC responses changed during animal running. As described previously, in trials where the mouse was stationary on the treadmill prior to photostimulation, IC units excited by vermis stimulation had a strong evoked response at an early and/or late latency.

The rasters of two representative IC neurons with excited evoked responses to vermis photostimulation are shown in Figures 4.1A and B. The overlaid heatmap depicts the animal's velocity during the photostimulation trials, where red indicates fast forward movement on the treadmill and white depicts the animal at rest. The cell in Figure 4.1A bad a two peak response to vermis photostimulation in trials when the animal was stationary. In trials when the animal was moving prior to or during the photostimulation, however, the evoked response was attenuated significantly at the time of each peak (Fig.

4.1C). This reduction in evoked response during running was found in cells of different response types. For example, the neuron depicted in Figure 4.1B had a long latency evoked response to vermis stimulation which was also attenuated during running (Fig. 4.1D). In addition, the baseline firing rate of the IC units increased while the animal was locomoting (Figs 4.1A, B). Running bouts were interspersed between periods of rest, when the vermis evoked response would return and the baseline firing is reduced (Figs 4.1A, B). These results indicate that ongoing animal locomotion was sufficient to modulate vermis influence on IC.

To compare the activity of IC neurons during animal running and rest, I classified running trials as ones where the animal's velocity exceeded 10 mm/s during the 300 msec time window preceding vermis photostimulation. If there were more than 20 running trials, I calculated the evoked and spontaneous firing rates of the IC units for both stationary and running trials. Across 24 IC units recorded from eight different mice (7 NOS1-ChR2; 1 L7cre; ArchT), excitatory vermis evoked responses were significantly reduced during animal running (Fig. 4.1E). Animal running also consistently increased the spontaneous firing rate of IC neurons (Fig 4.1F).

To investigate whether the decreased vermis-evoked firing rate was due to increased baseline activity, I calculated the difference in peak evoked firing rate in running and stationary trials, and it with the difference in baseline activity. The reduction in the vermis evoked response was also larger than the increase in spontaneous firing rate, indicating that the reduced effect of vermis photostimulation was not merely due to increased baseline firing (Fig 4.1G). Together, these results indicate that neuronal

responses to vermal input to the IC were reduced or gated off during animal movement and that the behavioral state of the mouse modulates IC physiological activity.

Running modulation of vermis evoked response was not caused by sound of animal movement

It is possible that animal and treadmill movement produced a sound which was responsible for the change in IC activity. However, the sound produced during animal movement was < 5 dB SPL above the background noise, and no significant high-frequency sounds were detected. The small amplitude of sound produced by running is unlikely to address the large changes in both evoked and baseline IC activity. However, some IC neurons have low auditory response thresholds so it is still possible that the sound of animal or treadmill movement could influence IC activity (Aitkin et al., 1994; Syka et al., 2000).

To further address potential auditory effects of animal movement, I ear plugged three NOS1-ChR2 mice to block their hearing and reduce auditory influence on IC activity. I confirmed that ear plugged mice had dampened hearing with field recordings in IC to auditory clicks and testing their behavioral response to loud sounds (e.g.: clapping behind the animal's head) before commencing with vermis photostimulation experiments. The example IC field recording in Figure 4.2A shows the average response IC to 70 dB SPL click when the mouse's ears were not plugged (in red). The following day, the mouse was anaesthetized and fitted with ear plugs, which were secured by external application a silicone elastomer (Kwik-Cast). I then performed field recordings

in IC while presenting auditory clicks of varying amplitudes. Figure 4.1A shows that the field recordings of louder clicks 80 dB SPL and below were smaller in the ear plugged mouse than responses to 70 dB SPL clicks when it was not ear plugged. Because the sound levels produced by treadmill movement were less than 60 dB SPL, it was likely that the ear plugs prevented the animal from hearing any movement produced sounds. Auditory field recordings were assessed each day in all animals to ensure no IC responses to clicks above 70 dB SPL.

In ear plugged mice, running also significantly reduced vermis evoked responses in the IC while increasing baseline activity (Fig 4.2, 4.3). There were multiple bouts of running between stationary periods during the photostimulation trial recordings as depicted in Figures 4.2B – E. In each running bout, the evoked responses following vermis photostimulation were reduced and the spontaneous activity increased; when the animal was stationary, the evoked and spontaneous activity returned to their respective previous levels (Figs 4.2B-E). The modulation effect of running on IC neuron evoked and baseline activity was similar in ear plugged mice as in non-ear plugged mice (Fig 4.3A, B). In fact, the vermis evoked response was more attenuated in ear plugged mice (Fig 4.3C, D). The increase in baseline activity remained the same between ear plugged and non-ear plugged conditions (Fig 4.3E). These results show the auditory consequences of animal locomotion is not likely to be what drove the modulation of IC activity to vermis photostimulation nor the increase in spontaneous neuronal firing.

Animal movement modulates IC response to auditory clicks

Different sensory brain areas, including the visual and auditory cortices have been shown to be robustly modulated by animal locomotion (Dadarlat & Stryker, 2017; Schneider et al., 2014). To my knowledge, no extensive study on the effect of locomotion on IC auditory responses has yet been conducted. To determine whether auditory stimulus-evoked responses in the IC was affected during movement, I presented auditory clicks during periods of rest and locomotion.

Similar to running's effect on vermis photostimulation response, auditory responses in the IC were attenuated, though still present, in trials when the animal ran. IC units that were excited by auditory clicks had a reduction in evoked responses during animal locomotion (Fig. 4.4). An example unit is shown in Figures 4.4A and B that had a brief, short latency excited response to a 70 dB SPL auditory click. The baseline firing rate was also increased during running.

Across 17 units that were excited by auditory clicks with sufficient running trials, most had a reduced response to click (14/17) and increased spontaneous firing rate (15/17) during running. The reduction in click evoked response during running was similar to the decrease in vermis photostimulation evoked activity of mice without ear plugs (Fig. 4.4E, F). As expected, the increase in baseline activity during running was also the same (Fig 4.4G). Taken together, locomotion changed the function of IC by increasing baseline activity and reducing its responsivity to both auditory stimuli and vermal signals.

Discussion

The auditory system is modulated by behavioral states. During active behavior, including locomotion, evoked responses in the auditory cortex and auditory thalamus are reduced (Schneider et al., 2014; Williamson et al., 2015; Zhou, Liang, et al., 2014). Responses to self-generated sounds are cancelled out at both the level of the dorsal cochlear nucleus (DCN) and the auditory cortex (Rummell et al., 2016; Singla et al., 2017). However, whether and how the auditory midbrain is affected by behavioral state is understudied and unclear.

In this study, I recorded IC neural responses in mice while they were running or stationary while head-fixed on a treadmill. I found that IC evoked responses to both vermis photostimulation and auditory clicks were reduced during running compared to when the mouse was at rest. Running also increased the spontaneous activity of IC neurons. The modulation of IC activity during running persisted in mice that were ear plugged.

Running gates off cerebellar vermis input to the IC

Both early and late evoked responses from vermis photostimulation were significantly suppressed in the majority of vermis-responsive IC neurons when the animal is locomoting (Figs 4.1, 4.2, 4.3). Running also increased the baseline firing of IC neurons (Figs 4.1-4), including spontaneous running outside of stimulus presentation trials. The reduction in evoked response was greater than the increase in spontaneous firing that also accompanies animal running (Fig 4.1G), indicating that the attenuated

evoked response was not entirely due to increased baseline firing. In the IC, running effectively increased the noise (spontaneous firing) while decreasing the signal amplitude (evoked response), thereby reducing vermis input.

The effect of locomotion on IC activity was not due to the sound produced by the mouse or treadmill motion. In order to reduce the effects of auditory feedback of treadmill movement on IC, I ear plugged mice and made sure that their hearing was impaired with IC field recordings and behavioral observation of the animal (Figs 4.2, 4.3). Similar to mice with unimpaired hearing, ear plugged mice had increased IC baseline activity during running (Fig 4.3B, E). Interestingly, the suppression of vermis evoked response was greater in ear plugged mice than mice that were not ear plugged (Figs 4.3A, C, D). This indicates that sound of treadmill movement may affect sensory gating of vermis input during running, albeit by reducing the suppression of vermis influence. The midvermis that was stimulated in these studies is known to receive auditory input (Huang & Liu, 1990; Snider & Stowell, 1944), so it is possible that the signal generated by vermis photostimulation could be slightly larger when there was auditory input. When the mice were ear plugged, the auditory influence on vermis were removed, thereby decreasing cerebellar output further.

It remains possible that ear plugged mice can perceive sounds of running via bone conduction (Stenfelt & Goode, 2005). Though unlikely, to completely exclude this possibility, mice could be chemically deafened or deaf mutant mice lacking Vesicular Glutamate Transporter 3 could be used (Seal et al., 2008). However, given the relatively small amount of noise the treadmill produces as well as the persistence of IC modulation during running when mice were ear plugged (Fig 3.2), the suppression of evoked

responses and increase in spontaneous activity were likely related to state-dependent shifts in behavior, and not auditory feedback.

Locomotion reduces the efficacy of auditory input to the IC

As a whole, the auditory input has been shown to be gated off throughout the auditory system during locomotion. Auditory cortex excitatory neuron responses to tones and thalamocortical terminal stimulation are reduced during animal movement (Schneider et al., 2014; Zhou, Liang, et al., 2014). Evoked responses are also reduced at the level of auditory thalamus (Williamson et al., 2015). In addition, the dorsal cochlear nucleus (DCN) has a "cerebellum-like" circuit that cancels out self-generated sounds produced by animal licking (Singla et al., 2017). In this study, I have found that animal running reduced IC neuronal responses to auditory clicks and increased spontaneous activity (Fig 4.4). Thus, many stages of the auditory system are gated off during animal locomotion, which seems most effectively engaged when the animal is still.

Though both vermis and auditory click evoked signals were significantly suppressed during running, responses in some neurons could still be measured (4.4E, F), meaning that some of the stimuli were robust enough to reach the IC. The auditory clicks used in these experiments were relatively loud (> 70 dB SPL) and also event-like due to their fast onset and offset. These sound properties probably make auditory clicks more likely to overcome the degraded IC signal-noise ratio caused by animal movement. In addition, loud, aversive sound information are carried by a non-canonical auditory circuit through the reticular formation (Zhang et al., 2017), which could potentially reach the IC

through another pathway. IC responses to quieter, longer latency sounds like noisebursts would probably be even more suppressed during animal locomotion.

It remains an open question whether inputs from other sensory modalities that the IC receives are also reduced when the animal runs. The IC receives visual information from the visual cortex and superior colliculus (Gruters & Groh, 2012a). Visual and saccade-related signals are found throughout the IC, particularly in regions with untuned auditory responses (Bulkin & Groh, 2011, 2012; Gruters & Groh, 2012a). Running increases the gain of visual cortex (V1) to visual stimuli (Dadarlat & Stryker, 2017; Niell & Stryker, 2010). Many visually-responsive neurons in the superior colliculus have shifts in spatial tuning and baseline activity during running (Ito, Feldheim, & Litke, 2017). Given the increased demands of the visual system during locomotion, it is possible that vision-related information remains ungated or is even enhanced in the IC during running.

The ECIC also receives somatosensory inputs to specific, neurochemically distinct modules (Aitkin et al., 1981; Gruters & Groh, 2012a; Jain & Shore, 2006; Zhou & Shore, 2006). It is possible that proprioceptive responses from locomotion suppresses auditory responses in IC (Aitkin et al., 1981; Aitkin & Zimmermann, 1978; Dehmel, Cui, & Shore, 2009).

Increased baseline activity in the IC may underlie and reinforce arousal and defensive behaviors

IC baseline activity increases during animal locomotion in photostimulation and auditory click trials (Figs 4.2, 4.4). I have also observed increases in spontaneous activity during running when the animal was not being presented with stimuli. Locomotion is correlated with general arousal; running entails a state of high arousal, though arousal does not necessitate locomotion (Busse et al., 2018; Vinck, Batista-Brito, Knoblich, & Cardin, 2015).

Electrical and pharmacological stimulation of the IC produces graded defensive behaviors of arousal, freezing, and running (Brandão et al., 2005, 1993, 1988). Electrical and chemical stimulation of IC arouses the animal in the form of desynchronized cortical electroencephalograms (EEG), hippocampal theta rhythms, and increased heart rate (Cabrera et al., 2013). In addition, bilateral acute IC inactivation with muscimol injections reduces wakefulness and increases sleep time (Cabrera et al., 2013). Pupillary dilation, a commonly used behavioral measure for arousal, is significantly correlated with spiking and local field potential activity in the IC of rhesus monkeys (Joshi et al., 2016). Brief microstimulation of the IC also evokes changes in pupil diameter (Joshi et al., 2016). The increased activity in IC during running can, in part, reflect arousal. Future studies measuring arousal (e.g.: pupillometry, EEGs) in conjunction with monitoring movement could help disassociate their respective effects on IC.

IC firing may also contribute to and reinforce the running behavior. Persistent sounds played near the mouse's head induces running, which is blocked by IC inactivation (Xiong et al., 2015). Optogenetic activation of auditory cortical inputs to the

IC shell region are sufficient to induce animal running, while optogenetic inhibition of corticofugal terminals in the IC suppresses running in response to persistent sounds (Xiong et al., 2015). Though there were no direct measurements of IC neural activity during the studies conducted by Xiong et al. 2015, my experiments show that the baseline activity of IC is increased when the mouse runs (Figs 4.1, 4.3, 4.4). It therefore seems likely that activation of IC during running is self-reinforcing and drives further flight behavior in the animal.

Differential functions of the visual and auditory systems during animal locomotion

When an animal is locomoting, it has different behavioral requirements than when it is stationary. If a mouse is at rest, a stimulus could entail figuring out whether to start moving or not in response. However, if that same mouse is subject to a stimulus while running, it no longer needs to decide whether to start moving, but instead merely where to go and whether to stop. Different sensory modalities appear to play different roles during animal locomotion. Visual stimuli can briefly arrest ongoing locomotion, a response that is mediated by corticotectal inputs from the visual cortex (V1) to the visual midbrain, the superior colliculus (Liang et al., 2015). Optogenetically activating V1 projection terminals in the superior colliculus is also sufficient to stop animal locomotion (Liang et al., 2015). By contrast, auditory cortical inputs to the IC produces running, and stimulation of these projections does not cause freezing (Xiong et al., 2015). In addition, Xiong et al. 2015 did not observe any sound-evoked freezing in their experiments.

The differing effects of visual and auditory stimuli on animal behavior during locomotion parallels how running modulates each respective sensory system. Locomotion produces a heightened response in the visual system, but reduces responsivity in the auditory system. These results imply that, during running, visual inputs are favored and perhaps more useful to the animal in responding to their rapidly changing environment, while auditory stimuli are less useful and gated off. Ongoing locomotion can produce self-generated sounds that do not provide the animal additional information that would determine its movement or whether to stop. Indeed, loud sounds originating from the external environment that still make it through the auditory system may signal greater danger from potential predators which are large and/or near, thereby inducing more running. Visual information could be more useful in helping an animal decide where to run or whether to stop and hide by determining where and how far a potential threat is, for example. This sensory dichotomy of evoked defense behaviors has been recently described for superior colliculus neurons: corticocollicular input from the auditory cortex drives flight, while input from the visual cortex instead produces freezing behavior (Zingg et al., 2016)

Interestingly, I observed that vermis photostimulation could also produce transient reductions in running speed. Though vermis signals were suppressed in the IC, projections from the downstream cerebellar output (fastigial) nucleus make divergent projections which can influence areas that are potentially not gated by animal locomotion, such as the reticular formation, motor cortices, and superior colliculus during animal running (Batton et al., 1977; Katoh et al., 2000; Noda et al., 1990; Snider & Maiti, 1976).

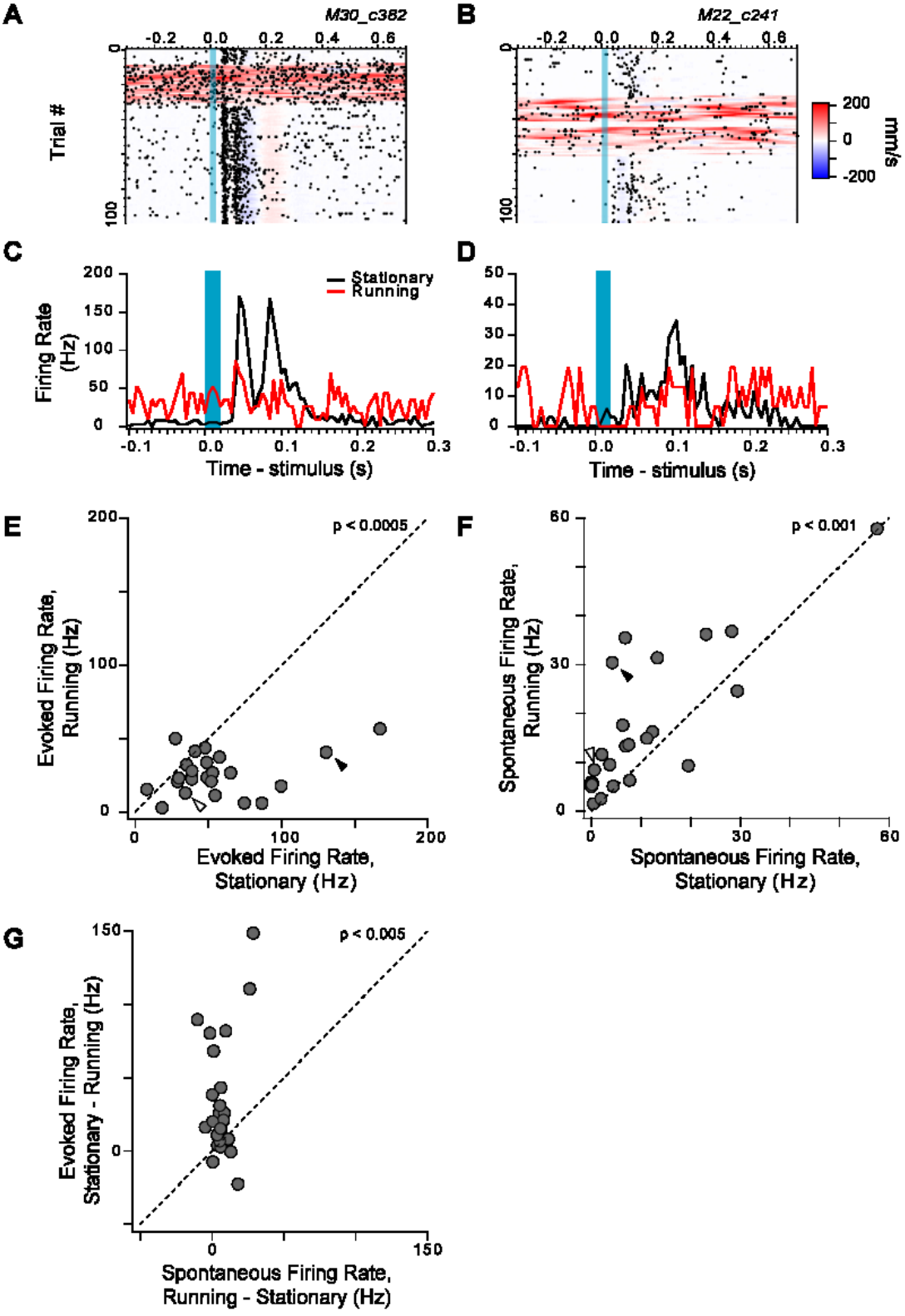
Potential circuit mechanisms for locomotor modulation of IC activity

Locomotor modulation of IC is likely due to influence from the cerebral cortex. Top-down projections from auditory cortex pyramidal neurons primarily target the shell regions (DCIC and ECIC) of the IC (Games & Winer, 1988; Schofield, 2010; Winer, 2006; Winer et al., 2002, 1998; Winer & Schreiner, 2005; Xiong et al., 2015). Before and during animal movement, fast-spiking inhibitory interneurons in the auditory cortex have increased firing rates, while putative excitatory neurons have decreased firing rates (Schneider et al., 2014). Premotor cortical (M2) neurons increase their activity during running and preferentially synapse onto PV+ inhibitory neurons in the auditory cortex (Nelson et al., 2013). Decreased auditory cortex activity during animal running could account for the increased baseline activity in IC; cooling of auditory cortex increases spontaneous activity in almost half of IC neurons in rats, though it also increased the magnitude of sound evoked responses (Popelář et al., 2016).

In the cerebellum, aversive stimuli, like air puffs and loud sounds, increase granule cell and interneuron activity as well as co-activation of Purkinje cells in vermis lobules IV/V (Ozden et al., 2012). During locomotion, however, these evoked responses are strongly suppressed and baseline activity of molecular layer interneurons are increased (Ozden et al., 2012). Optogenetic activation of molecular layer interneurons could have reduced effect on the synchronized inhibition of PCs. Together, this suggests that vermis activity is gated during animal running, and that event-related signals from the cerebellum are weaker and/or less likely to be broadcast to the rest of the brain.

Running affects neuromodulator neurons of the brain. In particular, cholinergic neurons in the pontomesencephalic tegmentum (PMT), comprised of the laterodorsal tegmentum (LDTg) and pedunculopontine tegmentum nuclei (PPTg), are the sole source of cholinergic input to the IC. Stimulation of these cholinergic nuclei produces arousal (Brudzynski, 2014; Curro Dossi et al., 1991; Steriade, Dossi, Pare, & Oakson, 1991; Steriade, Paré, Datta, Oakson, & Curró Dossi, 1990). The PPTg in particular is part of the mesencephalic locomotor region, and has increased activity during locomotion, voluntary movements, and saccades (Jenkinson et al., 2009). In addition, electrical and optogenetic stimulation of the PPTg produces locomotion (Jenkinson et al., 2009; Xiao et al., 2016). Acetylcholine increases responses of IC neurons to repetitive sounds as well as baseline activity, particularly in neurons with medium responsivity to repetitive sounds (Ayala & Malmierca, 2015). Running could increase acetylcholine release in the IC and change baseline firing rates. Evoked responses to auditory clicks and vermis photostimulation may not have been affected due to the relatively low rates of stimulus presentation in my experiments.

Figures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0.2
0.4
0.6
Trial #
0
100
B
M22_c241
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0
-200
mm/s
C
Stationary
Running
Firing Rate (Hz)
200
150
100
50
0
-0.1
0.0
0.1
0.2
0.3
Time - stimulus (s)
D
50
40
30
20
10
0
Time - stimulus (s)
E
p < 0.0005
Evoked Firing Rate, Running (Hz)
Evoked Firing Rate, Stationary (Hz)
F
p < 0.001
Spontaneous Firing Rate, Running (Hz)
Spontaneous Firing Rate, Stationary (Hz)
G
p < 0.005
Evoked Firing Rate, Stationary - Running (Hz)
Spontaneous Firing Rate, Running - Stationary (Hz)

Figure 4.1: Running attenuates inferior colliculus response to cerebellar photostimulation and increases spontaneous activity

A, B. Example rasters of representative vermis-responsive IC neurons overlaid with treadmill velocity heatmaps during photostimulation trials. Periods of rest are in white, while running is red.

C, D. Average firing rate of neurons in (A) and (B) to vermis photostimulation during running and stationary trials.

E. Maximum evoked firing rate to vermis photostimulation during rest versus running trials ($n = 24$ units, $p < 0.0005$, paired two-tailed Wilcoxon Signed Rank Test). Black arrowhead, neuron in (A) and (C). White arrowhead, neuron in (B) and (D).

F. Spontaneous firing rate preceding photostimulation during rest vs running ($n = 24$ units, $P = 0.001$, two-tailed paired Wilcoxon Signed Rank Test). Black arrowhead, neuron in (A) and (C). White arrowhead, neuron in (B) and (D).

G. Comparison of difference in max evoked and baseline changes between stationary and running trials ($n = 24$ units, $P = 0.005$, two-tailed paired Wilcoxon Signed Rank Test).

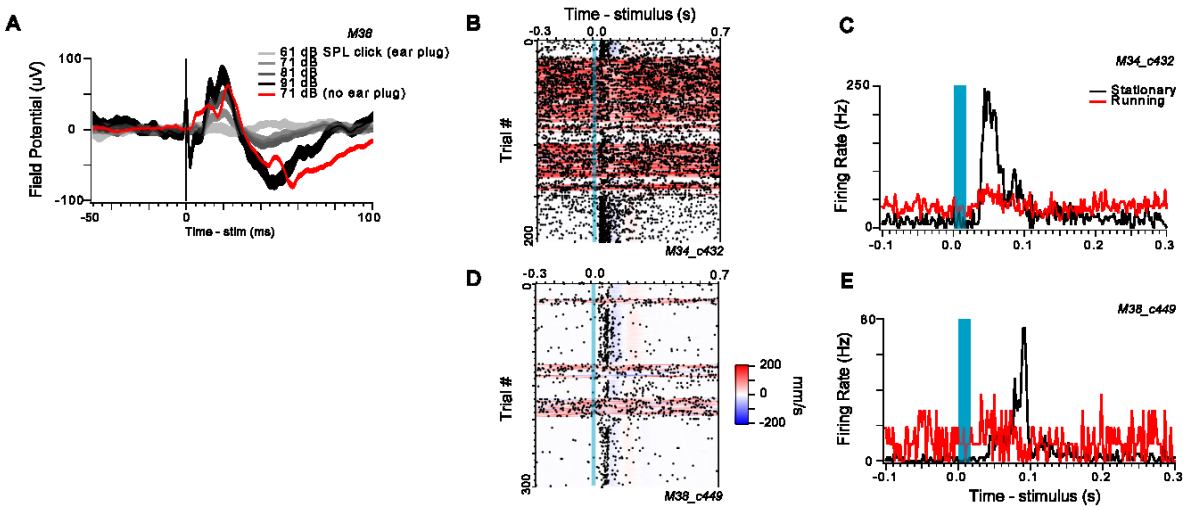

Figure 4.2: Representative IC neuron responses to vermis photostimulation in ear plugged mice during running and stationary trials

A. Example IC field potential recordings in the same mouse to auditory clicks in ear plugged (black) and non-ear plugged (red) to confirm ear plugs attenuate animal hearing. The ear plugged recordings were conducted one day after the non-ear plugged recordings.

B, D. Representative rasters of IC neurons in different ear plugged animals during vermis photostimulation trials. Running bouts indicated in red.

C, E. Average firing rates of neurons in (A) and (C), respectively, to vermis photostimulation during running and stationary trials.

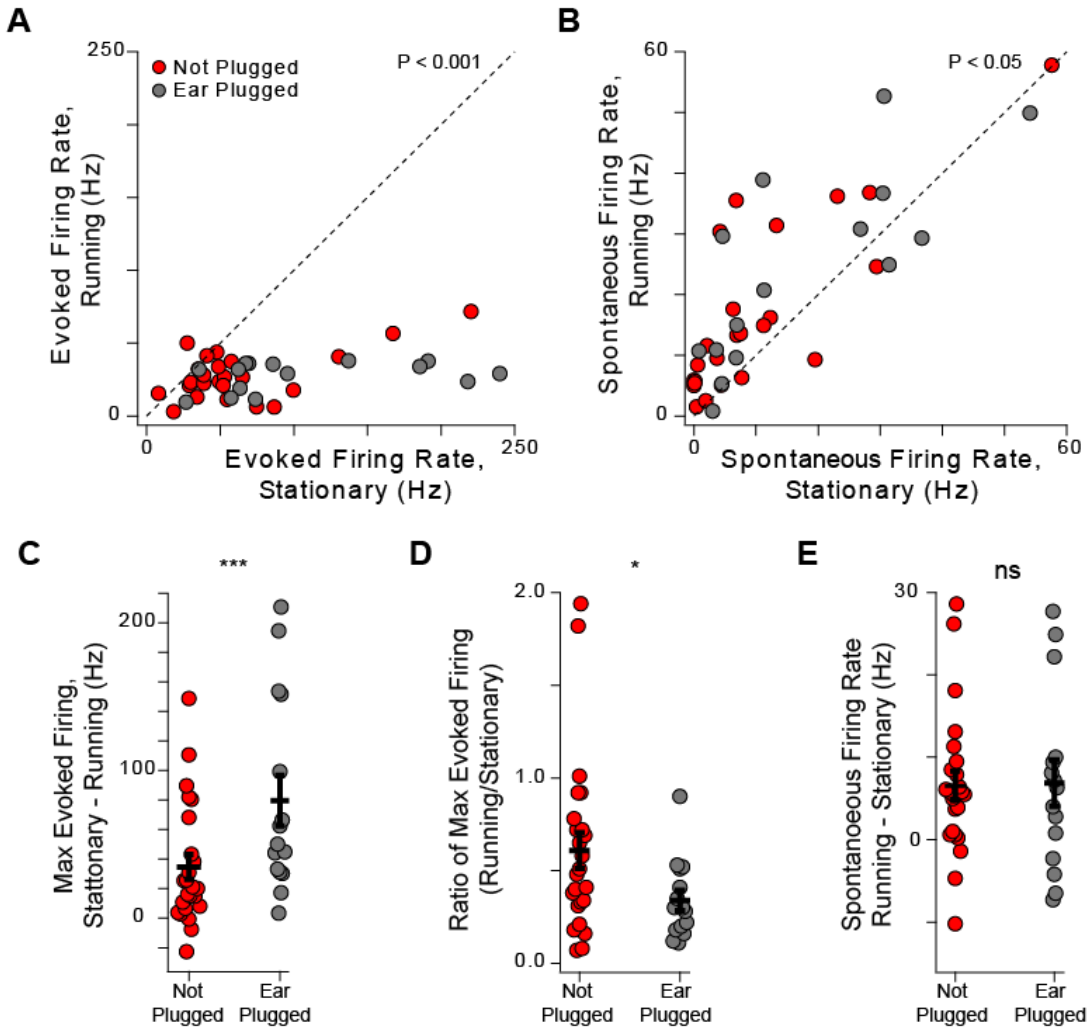

Figure 4.3: Running modulates IC response to vermis photostimulation in ear plugged mice

A. Maximum evoked firing rate to vermis photostimulation during rest versus movement in ear plugged (grey) and non-ear plugged (red) mice (n = 15 cells, P = 0.001 paired two-tailed Wilcoxon Signed Rank Test).

B. Spontaneous firing rate preceding photostimulation during rest vs movement in ear plugged (black) and non-ear plugged (red) mice (n = 15 cells, P < 0.05, paired two-tailed Wilcoxon Signed Rank Test).

C. Absolute change in maximum evoked firing rate between photostimulation stationary and running trials in non-ear plugged and ear plugged mice.

D. Ratio of evoked response to vermis photostimulation in running trials compared to stationary trials in non-ear plugged and ear plugged mice.

E. Difference in spontaneous firing rate change in running trials from stationary in non-ear plugged and ear plugged mice. ***P < 0.001; *P < 0.05; ns, not significant.

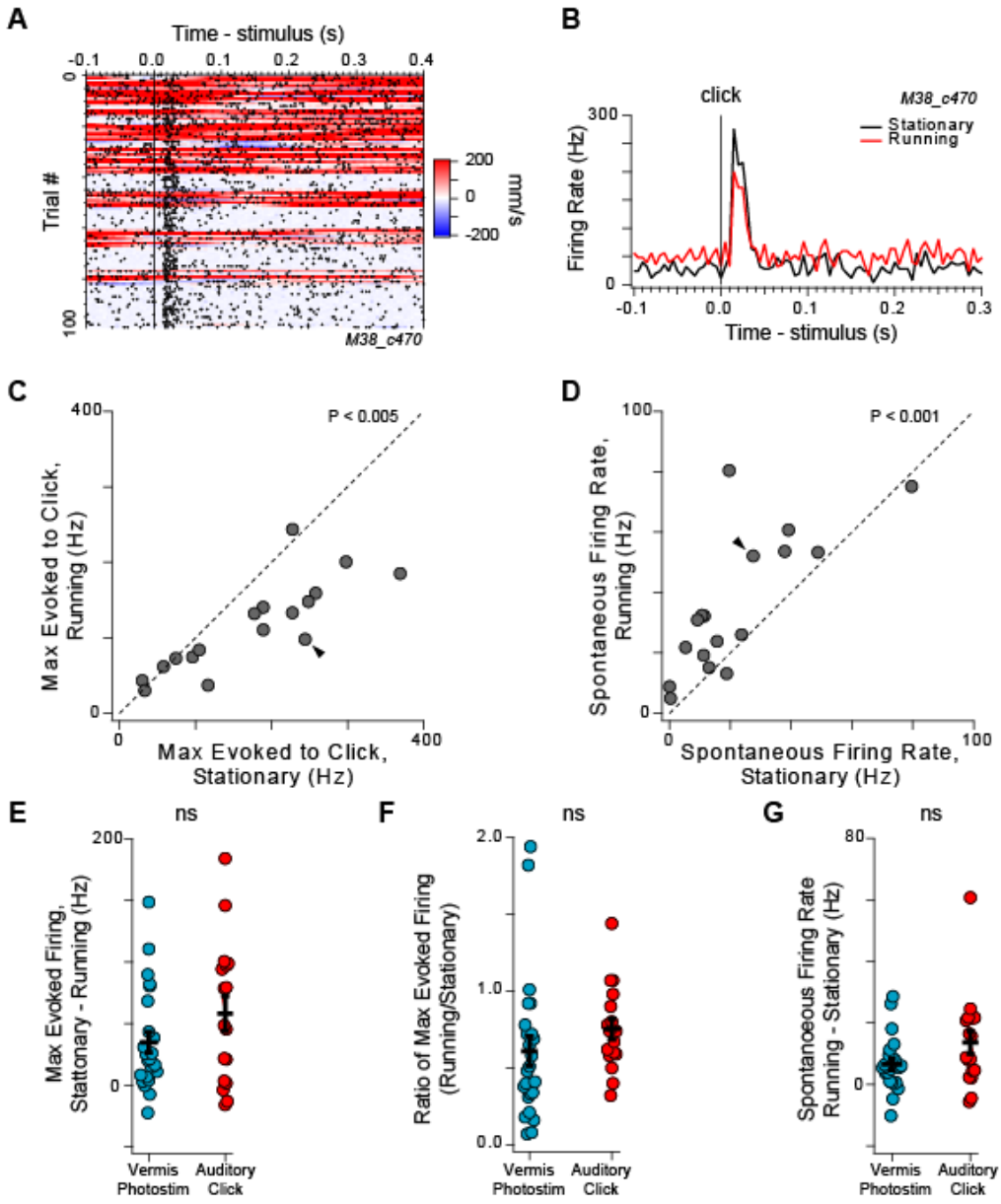

Figure 4.4: Locomotor state modulates IC responses to auditory clicks

A. Example IC unit rasters in trials with 70 dB SPL auditory clicks during animal movement (red) and rest (white).
B. The average firing rate of same unit in (A) to auditory click during running and stationary trials.
C. Maximum evoked firing rate to auditory clicks during rest versus movement (n = 17 units, $P < 0.005$, paired two-tailed Wilcoxon Signed Rank Test). Arrowhead denotes example unit in A and B.
D. Spontaneous firing rate preceding auditory clicks during rest vs movement (n = 17 units, $P < 0.001$, paired two-tailed Wilcoxon Signed Rank Test).

E. Comparison of photostimulation and auditory click evoked response attenuation in absolute firing rate between stationary and running trials.
F. Comparison of ratio of evoked firing rate to photostimulation and auditory click between stationary and running trials.
G. Comparison of changes in baseline activity prior to vermis photostimulation and auditory click presentation in stationary and running trials. ns, not statistically significant.

Chapter 5. Conclusions and Future Directions

In this thesis, I have attempted to shed light on how the auditory system can get the necessary contextual information to detect and produce rapid behaviorally appropriate responses to salient stimuli. To tackle this question, I investigated the functional link between two brain areas that have more in common than has been appreciated. My experiments demonstrate that the cerebellar vermis robustly modulates activity throughout the inferior colliculus (IC) and may be providing an alerting signal. My work also shows that different modalities of salient stimuli produce similar behavioral responses. Auditory and vermis stimulation both could produce short latency startle movements as well as a longer latency movement that is typically not described in the startle behavior literature. In addition, I discovered that IC activity was modulated by animal locomotor state, which has previously not been studied and fills a gap in the auditory literature.

Almost seventy years ago, the neuroscientist Ray Snider observed that the cerebellum is the "great modulator of neurologic function" (Snider, 1950). As is often the case, though my work expands our knowledge about the role the cerebellum plays in modulating brain activity in the IC, it ultimately raises more questions than it answers. Below, I will highlight the main conclusions I draw from my studies, as well as discuss the implications of my results and the new questions that are brought forth.

The cerebellar vermis is functionally connected to the IC

In Chapter 2, I demonstrated that the cerebellum has a robust, polysynaptic influence on the IC. When Purkinje cells (PCs) in the cerebellar vermis were directly or indirectly silenced, the majority of neurons in the IC had modulated neural activity. Previous studies describe a modifying of auditory responses caused by cerebellar stimulation (Crispino & Bullock, 1984; Velluti & Crispino, 1979), whereas my results show that vermis stimulation can alter IC firing *sans* sound.

Notably, my findings also indicate that the cerebellar vermis forms a reciprocal circuit with the IC. The IC has known projections to the dorsolateral pontine nucleus (DLPN), whose neurons send mossy fibers to the auditory receiving areas in the vermis and produce the sound-related signals (Aitkin & Boyd, 1978; Huang & Liu, 1990; Huang et al., 1991; Snider & Stowell, 1944). It is of interest to note that different populations of pontine neurons receive inputs from the IC versus the auditory cortex; the mossy fibers ascending to the midvermis is the target of IC auditory information, while the paraflocculus (a cerebellar structure involved in smooth pursuit eye movements) receives mossy fiber inputs carrying signals from the auditory cortex (Azizi et al., 1985, 1981; Azizi & Woodward, 1990). Intriguingly, the auditory responses in paraflocculus are sustained, akin to the smooth pursuit eye movements it produces, while the responses to sound in the midvermis are transient like the saccades it generates (Azizi et al., 1985). From this, I suspect that the auditory responses measured in both cerebellar regions can modulate the eye movements they respectively produce. Thus, midvermis (lobules VI/VII) receives auditory information from the IC, and stimulation of this area produces evoked signals in the IC, establishing a potential feedback loop.

How does the vermis signal make its way to the IC?

Given the robust evoked responses produced by vermis photostimulation as well as the pontocerebellar input from IC, how does the vermis signal get relayed to the IC? In experiments not presented in this dissertation, I tried to answer this question by making anterograde tracer injections into the fastigial nucleus (FN), which is the cerebellar output nucleus of the vermis, along with retrograde tracer injections into the IC. These anatomical experiments re-affirmed previous studies in finding that there are no direct connections between the FN and IC, but revealed several potential polysynaptic circuits that could be carrying the vermis photostimulation signal.

The caudal FN, which is innervated by PCs in vermal lobules VI/VII, makes projections onto cholinergic and non-cholinergic neurons (as determined by choline acetyltransferase immunostaining) in the laterodorsal tegmentum (LDTg) and pedunculopontine tegmentum (PPTg). Together, these two midbrain nuclei provide the sole cholinergic input to the IC (Schofield, Motts, & Mellott, 2011); my retrograde tracer injections confirmed that neurons in the LDTg did indeed send projections to the IC. Thus, the LDTg could be one candidate intermediary brain region connecting the FN to the IC. However, the vermis evoked responses I observed in IC were both robust as well as consistent in their response latencies, something that is unlikely to be produced by neuromodulatory action.

A thalamocortical circuit therefore seems more likely to carry the vermis alerting signal. My anatomical experiments corroborate previous studies showing that the FN densely innervates the thalamic subnuclei that project to motor cortices (M1 and M2) as well as prefrontal cortical regions (like the medial prefrontal cortex) (Middleton & Strick,

2002; Steriade, 1995; Watson, Becker, Apps, & Jones, 2014). These cortical regions are both known to project to auditory cortices (Nelson et al., 2013; Schneider et al., 2014), which in turn give strong, top-down feedback down to the IC, predominately in the ECIC and DCIC (Winer et al., 1998). In addition, I found that IC neurons inhibited by vermis photostimulation were also inhibited by auditory clicks, which suggests that the circuit connecting the vermis and IC includes part of the auditory circuits.

The density of FN input to the thalamus in conjunction with the number of layer V and VI neurons in the auditory cortices that project to the IC could account for the widespread, robust evoked responses that are produced from vermis photostimulation (Winer, 2006). That this circuit would span many synapses could account for the relatively long delay between vermis photostimulation onset and evoked responses in the IC (> 30 msec); by my count, there are at least six synapses that separate the cerebellar molecular layer interneurons being optogenetically stimulated and the IC neurons that I recorded.

I performed several pilot experiments to test whether blocking neural activity in candidate intermediary brain regions between vermis and IC would also block the evoked response. However, these inactivation studies were not conclusive; optogenetic (using Gad2-cre; ai27 transgenic mice) and pharmacological (using muscimol and lidocaine) blockade of neural activity in the ipsilateral auditory cortex or M2 cortex did not consistently block vermis-evoked responses in the IC. One reason for this could be the interconnectivity of the different IC subdivisions and hemispheres (Saldaña, Feliciano, & Mugnaini, 1996; Saldaña & Merchań, 1992), which could compensate for unilateral

inactivation. I hypothesize that if the auditory cortices were bilaterally inactivated, IC neuron responses to vermis stimulation would be suppressed, if not abolished.

The cerebellar vermis sends an event-related alerting signal that reaches the IC

Why would the IC be functionally connected to the vermis? My findings from Chapters 2 and 3 suggest that the vermis transmits an alerting signal to the IC, which could make use of that signal in its roles modulating sensory signals and producing acousticomotor, defensive behaviors.

When a salient stimulus occurs, animal must attend to, process, and ultimately decide how to appropriately respond to it. The results presented in Chapter 3 show that different types of salient stimuli (auditory and vermal in origin) produce movements in response. Following loud, unpredictable auditory clicks, mice produce short latency startle responses as has been well-documented in the startle literature (Koch, 1999; Lauer et al., 2017; Yeomans & Frankland, 1996; Yeomans et al., 2002). However, I observed that following acoustic stimuli presentation mice also produce a longer latency movement, which is not described in previous research studying startle behavior. Crucially, I believe that this later movement is evidence that animals still respond to and make preparatory movements following salient, but sub-startle stimuli (Novembre et al., 2018).

The cerebellar vermis plays an important role in alerting the rest of the brain to these salient stimuli. In my optogenetic experiments, PCs are synchronously silenced, disinhibiting downstream FN neural activity thereby driving a cerebellar output to the rest of the brain. Coordinated pauses in firing is a hallmark of PC responses to startling or

surprising stimuli. The vermis photostimulation used approximately mimics the brief, synchronized pause in PC activity following a complex spike, caused by input from neurons in the inferior olive (IO). These electrically-coupled IO neurons are activated by errors produced by the sensory consequences of movement, such as retinal slippage from a saccade target, and coordinate firing in populations of PCs (Azizi & Woodward, 1987).

What would more naturalistically cause synchronized pausing of PCs and increased cerebellar output during animal behavior? Unexpected stimuli in general could produce these teaching signals, PC inhibition, and subsequent cerebellar output signals. Interestingly, the cerebellar vermis is activated by painful stimuli in humans and animals (Borsook, Moulton, Tully, Schmahmann, & Becerra, 2008; Moulton et al., 2011; Moulton, Schmahmann, Becerra, & Borsook, 2010). Stimuli producing pain are particularly salient and effective at modifying animal brain state and behavior. Animals need to pay attention to salient stimuli – particularly unexpected ones – in order to adapt their behaviors and survive. Unexpected changes in the environment may require the animal to change in turn, in both its brain state and behavioral output.

Indeed, as described in Chapter 3, I observed that auditory clicks sometimes caused animal twitches with accompanying IC activity. These observations were serendipitous, and future experiments could be designed to increase the probability of an auditory click-evoked movement. For example, substartle auditory clicks could be presented in an unpredictable manner (at random or pseudorandom, long inter-trial intervals) while recording IC neural activity and monitoring movement of the treadmill. Unpredictable stimuli are more salient, and are more likely to produce motor responses than regularly presented stimuli, so this experimental design would permit easier

comparison of IC responses during sound-evoked movement trials with responses in non-movement trials. I hypothesize that IC neurons with alerting and/or motor corollary signals to vermis photostimulation would also have those responses to unexpected auditory clicks, particularly when the animal makes a late, preparatory movement. That is, if a given IC cell has an early alerting response to vermis photostimulation (with response latency of 30 – 60 msec), I would expect it to also have increased firing at a similar latency with higher response probability during trials with preparatory movements. If the IC neuron has late evoked firing during vermis photostimulation trials with animal late movement, I would predict that the cell would also have increased firing during auditory click trials where the mouse makes a late movement.

In addition, I hypothesize that the alerting signal measured in the IC would be produced in response to different modalities of stimuli, provided that they are salient and behaviorally relevant to the animal. To test this, airpuffs (a type of salient, somatosensory stimulus) could be delivered at long, random intervals to the mouse's back while recording IC neural activity. If alerting signals in the IC are multimodal, then I would expect that neurons with alerting signals to vermis photostimulation would produce an early evoked response to auditory clicks and airpuffs. Similar to my findings with vermis photostimulation, the auditory- or airpuff-evoked firing in IC would not causally relate to animal movement, but its response probability may be positively correlated. The cerebellar vermis receives somatosensory and proprioceptive inputs (Huang & Liu, 1990; Ishikawa, Shimuta, & Häusser, 2015; Restuccia, Marca, Valeriani, Leggio, & Molinari, 2007; Saab, Garcia-Nicas, & Willis, 2002; Snider & Stowell, 1944), and may drive cerebellar output in response to unexpected tactile stimuli to the IC.

Interestingly, the response latency of the vermis-evoked alerting signal in IC corresponds with the timing of the N1 auditory event-related potential (ERP), a correlate of auditory feature- and event-detection (Horváth, 2015). Because the N1 is mediated by the auditory cortex, the vermis-evoked IC alerting signal may also be mediated via thalamocortical circuits. EEG and LFP recordings in IC conducted simultaneously during vermis photostimulation and unpredictable acoustic stimuli presentation would help determine whether and how the cortical N1 signal is related to the putative alerting signal in IC.

As the English electrophysiologist, Sir Charles Sherrington, observed: "The motor individual is driven from two sources. The world around it and its own inner world within. Its activity is also partly operated by nervous activity arising spontaneously within the nervous centres themselves" (Sherrington, 1940). The cerebellar vermis can serve to bridge the worlds within and without by alerting the rest of the brain, including the IC, in order to produce a rapid, coordinated response to the changes that arise.

Does the cerebellar vermis modulate auditory processing?

Given that vermis stimulation can robustly drive activity in the IC, does auditory processing get modulated as well? Previous studies investigating the effects of cerebellar stimulation on the auditory system, including the IC, found a modulation of responses to auditory stimuli (Crispino & Bullock, 1984; Velluti & Crispino, 1979).

In a few preliminary experiments, I presented trials where vermis photostimulation preceded auditory clicks of different intensities, interleaved with trials presenting the vermis photostimulation and the auditory clicks alone. In these

experiments, I varied the intensities, durations, and interstimulus intervals while recording from IC neurons. My results were not conclusive and did not show a reliable effect of cerebellar stimulation on IC responses to auditory clicks. In a few neurons, however, vermis photostimulation suppressed the response to auditory clicks. Though very preliminary, these results align with older studies, which predominately found a suppressed auditory response when the vermis was stimulated (Crispino & Bullock, 1984; Velluti & Crispino, 1979). Testing different auditory stimuli (e.g.: noisebursts), bilaterally photostimulating vermis, and varying interstimulus intervals might increase the likelihood of vermis-mediated auditory response modulation in the IC.

These results are suggestive of a neural mechanism that could contribute to a prepulse inhibition of startle responses to auditory stimuli, as both the IC and cerebellar vermis are important for modifying the startle response (Koch, 1999; Lauer et al., 2017; Leaton & Supple, 1986). As was described in Chapter 3 of this dissertation, unexpected, loud auditory clicks and consistently presented vermis photostimulation could evoke an early startle movement. If the vermis produces an alerting signal, I hypothesize that vermis photostimulation could serve as a prepulse stimulus that reduces startle behavior. This could be experimentally tested by measuring the movements produced by unexpected (> 10 seconds inter-trial interval), loud auditory clicks or strong vermis photostimulation in trials with or without a preceding vermis photostimulation that is sub-startle threshold (produced either by decreasing light intensity and/or stimulation duration). I hypothesize that vermis photostimulation presented 50 – 200 milliseconds before the startling stimulus would inhibit the startle movement, while much shorter lead

times (< 15 msec) would facilitate the movement, akin to what is observed when acoustic stimuli are used as prepulses (Koch, 1999; Lauer et al., 2017).

The response characteristics of IC neurons are heterogenous

As described in Chapter 2, vermis evoked responses were found distributed throughout the IC. Though there was some topography to single units with different response types to vermis photostimulation – more two peak and late responses laterally, a preponderance of early responses medially, and inhibited responses ventrally – neurons exhibiting different response properties could be recorded in the same recording site. Similarly, neural responses to auditory clicks were also heterogeneous in IC cells that were excited by vermis photostimulation. The heterogeneity of responses is likely due to the diverse inputs a given IC subregion or neuron receives, including different sources of ascending auditory input, descending cortical input, and intracollicular connections (Winer & Schreiner, 2005). Heterogeneous responses in IC are hypothesized to drive further feature extraction and efficient encoding of complex stimuli (Holmstrom, Eeuwes, Roberts, & Portfors, 2010).

In order to better understand the function of the vermis-evoked alerting or motor corollary discharge signal, it is necessary to know the projection targets of these neurons. To address this question, juxtacellular recording experiments could be conducted to assess IC activity to vermis photostimulation and to fill each cell with a tracer, which would permit the recovery of the recorded cell's exact location, axonal projection destinations, and neurochemical identity (Joshi & Hawken, 2006; Tang, Brecht, & Burgalossi, 2014). Alternatively, injections of retrograde viral tracers carrying ChR2 into

IC target regions could allow for "opto-tagging" of IC neurons during recordings, wherein IC neurons projecting to the target region would identified by responsivity to optogenetic activation (Cohen, Haesler, Vong, Lowell, & Uchida, 2012).

Heterogeneity of IC responses was also described in previous studies investigating visual- and visuomotor-responses, which were recorded throughout the IC though found to be more robust in untuned auditory areas (Bulkin & Groh, 2012; Porter et al., 2007). IC cells that had modulated auditory responses relative to animal eye position are similarly found throughout the structure, with stronger response patterns again in untuned auditory locations (Groh et al., 2001). It is possible that these responses facilitate IC involvement in orienting behaviors to sound sources. Given the vermis's role in saccadic eye movements (Kheradmand & Zee, 2011; Ohtsuka & Noda, 1991), and the increased responses of vermis cells to auditory stimuli located directly in front of the animal (Aitkin & Boyd, 1975), testing whether vermis-responsive IC neurons are also modulated by front-facing and/or moving auditory stimuli would be illuminating for understanding the functional relationship of these two brain structures.

If the vermis drives an alerting signal in IC, it would be interesting to test whether vermis-responsive neurons modulated are also more likely to be responsive to naturalistically salient stimuli, like vocalizations (Holmstrom et al., 2010; Huffman & Henson, 1990; Portfors, 2007). The IC contains neurons that are responsive to sounds of social vocalizations, though there is heterogeneity in vocalization selectivity (Holmstrom et al., 2010). In addition, neurons with similar characteristic frequencies respond differently to the same vocalization (Holmstrom et al., 2010). Notably, IC neurons that respond better to conspecific vocalization than to white noise and pure tones are more

commonly found in the ECIC and DCIC (Aitkin et al., 1994; Portfors & Roberts, 2014), similar to distributional biases for vermis-, visual-, and saccade-related responses (Bulkin & Groh, 2012; Gruters & Groh, 2012a; Porter et al., 2007). Thus, recording and comparing IC responses to audio clips of mouse vocalizations and vermis photostimulation may reveal an overlap in functional cell types responsive to different salient, behaviorally relevant stimuli.

Behavioral state gates cerebellar and auditory inputs to the IC

The results in Chapter 4 show that animal locomotion modulates IC activity by attenuating auditory and vermis signals, and increasing spontaneous activity. To my knowledge, this study is the first time that locomotor modulation of IC activity has been investigated. Similar to the medial geniculate body (Williamson et al., 2015) and the auditory cortices (Nelson et al., 2013; Schneider et al., 2014), IC responses to acoustic stimuli were suppressed during running. I found that vermis evoked signals were also attenuated and IC baseline activity was increased. These results indicate that locomotion changes brain state, including in the IC, based on the changing behavioral needs of animal.

It is an open question as to how locomotion is gating IC input. Running could activate cholinergic neurons in the laterodorsal tegmentum (LDTg) and pedunculopontine tegmentum nuclei (PPTg), increasing cholinergic tone in the IC (Jenkinson et al., 2009; Xiao et al., 2016). M2 neurons have been found to preferentially synapse onto and excite inhibitory neurons in the auditory cortex during locomotion, thereby decreasing activity

in excitatory neurons (Schneider et al., 2014); top-down input from the auditory cortices to the IC could in turn modulate activity.

Implications of cerebellar influence on the IC for neurological disorders

The location in the cerebellum I stimulated – vermis lobules VI/VII – wears many metaphorical hats and plays many different functions. It is the oculomotor vermis, important in saccadic eye movements (Noda & Fujikado, 1987; Voogd & Barmack, 2005). It is Snider's "teleceptive" receiving area, where auditory and visual signals are found alongside somatosensory ones (Altman et al., 1976; Huang & Liu, 1990; Jen & Schlegel, 1980; Snider & Stowell, 1944). It is also the "limbic cerebellum", involved with Jeremey Schmahmann's cerebellar cognitive affective syndrome (CCAS) and the locus of a veritable cornucopia of neuropsychiatric disorders (Hoppenbrouwers, Schutter, Fitzgerald, Chen, & Daskalakis, 2008; Mosconi, Wang, Schmitt, Tsai, & Sweeney, 2015; Schmahmann, 2000, 2004; Shakiba, 2014; Tsai et al., 2012). As Snider would remark in a 1958 Scientific American article dedicated to the cerebellum: "It becomes increasingly evident that if 'integration' is a major function of this organ, trips into the realm of mental disease may cross its boundaries more frequently than the guards in the sanitariums suspect" (Snider, 1958). Given that the cerebellar vermis modulates the IC, and that both structures help animals produce appropriate behaviors to stimuli, what occurs when this modulation goes awry?

One possible outcome could be overmodulation of IC activity by the vermis. Tinnitus and hyperacusis often develop due to age- and noise-related hearing loss (Roberts et al., 2010; Sedley, 2019), and are associated with sound-evoked hyperactivity

and increased spontaneous firing in the IC (Xiong et al., 2017). In addition, the cerebellar vermis has recently been implicated in playing a role in tinnitus development following hearing loss (Bauer, Wisner, Sybert, & Brozoski, 2013; Chen et al., 2017; Feng et al., 2018). Cerebellar output could potentially increase the gain on central auditory activity in order to increase the acoustic signal after damage to the peripheral auditory system (Bauer et al., 2013). This vermis-mediated gain increase could compensate for hearing loss, but at the potential cost of amplified neural background noise. It would be elucidating to test whether mice with hearing loss, either due to age or noise exposure, have changed evoked response amplitudes to vermis photostimulation in the IC.

In addition, schizophrenia and autism spectrum disorder are both associated with cerebellar atrophy or damage to the limbic vermis of lobules VI/VII (Kern, 2002; Schmahmann, 2004; Schmahmann et al., 2007; Tsai, 2016; Tsai et al., 2012). In fact, damage to the vermis during development is the largest single nonheritable risk factor for autism (Wang, Kloth, & Badura, 2014). Interestingly, both schizophrenia and autism can manifest with auditory-related deficits. Schizophrenia is a psychiatric disorder that can include positive symptoms of auditory hallucinations. Autism is characterized by impairments in social interaction, but also marked with problems with attention, orientation, and responses to sensory stimuli (Kern, 2002; Millin et al., 2018; Noel, Stevenson, & Wallace, 2018; Orekhova, Stroganova, & Poljac, 2014). People with autism may become fixated on certain stimuli to the exclusion of more important ones (Kern, 2002). It is estimated that most children with autism suffer from sensory hypersensitivity, including hyperacusis (Sinha et al., 2014). Given the findings in my study, it seems likely that the cerebellar vermis, as the "great modulator of neurologic

function", and its interactions with the IC plays a role in these disorders and may be a prime target for novel treatments in the future.

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Curriculum Vitae

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Education

Johns Hopkins University, Baltimore, MD, USA **2013 – present**
Ph.D. candidate in Neuroscience, expected May 2019

Harvard University, Cambridge, MA, USA **2008 – 2012**
A.B. *cum laude* with high honors in Neurobiology in the Mind/Brain/Behavior interdisciplinary track

Research Experience

Graduate Research Assistant, Johns Hopkins University **2015 – present**
Thesis project: *"The cerebellar vermis robustly modulates neural activity in the inferior colliculus"*
Thesis Advisor: Sascha du Lac, Departments of Neuroscience, Neurology, and Otolaryngology

Graduate Rotation Student, Johns Hopkins University **2014 – 2015**
Advisor: Jeremiah Cohen, Department of Neuroscience

Visiting Scientist, Max Planck Institute of Neurobiology **2012 – 2013**
Advisor: Hiromu Tanimoto
Sponsored by a scholarship from the German Academic Exchange Service

Undergraduate Research Assistant, Harvard Medical School **2008 – 2012**
Advisors: Edward Kravitz and Mireya Nadal-Vicens

Awards and Honors (selected)

International Neuroethics Society Student/Postdoc Essay Contest Finalist **2018**
RehabMart Scholarship Winner **2018**
Graduate Student Association's Student Group Leader of the Year **2018**
Baltimore Science Slam Winner, "Most Creative Talk" by audience vote **2016**
National Science Foundation Graduate Research Fellowship Program
Honorable Mention **2014, 2015**
German Academic Exchange Service Graduate Study Scholarship **2012 – 2013**
Alex Booth Fund Fellowship **2012 – 2013**
Harvard College Program for Research in Science and Engineering Fellow **2011**
Mary Gordon Roberts Summer Mind, Brain, and Behavior Fellowship **2011**

Herchel-Smith Summer Undergraduate Research Fellowship **2010**
Harvard College Research Program Scholarship **2009 – 2010**

Publications

Yamagata N, Ichinose T, Aso Y, Plaçais PY, Friedrich AB, **Sima RJ**, Preat T, Rubin GM, H Tanimoto. (2015). Distinct dopamine neurons mediate reward signals for short- and long-term memories. ***Proceedings of the National Academy of Sciences***, *112*(2), 578-583.

Presentations

Sima RJ, Kodama T, Fujita H, du Lac S (2018) The cerebellum robustly modulates neural activity in the inferior colliculus. Society for Neuroscience conference. [Poster]

Sima RJ, Kodama T, Fujita H, du Lac S (2018) The cerebellum robustly modulates neural activity in the inferior colliculus. Johns Hopkins University Neuroscience Retreat. [Poster]

Sima RJ, Kodama T, Fujita H, du Lac S (2018) The cerebellum robustly modulates neural activity in the inferior colliculus. Johns Hopkins University Center of Hearing and Balance Trainee Seminar. [Talk]

Matney CJ, **Sima RJ**, Carlson J, Cairns L, Wood K, Pham D (2018) Johns Hopkins Science Policy Group: Training scientists to be effective advocates and communicators. Experimental Biology conference [Poster]

Fujita H, **Sima RJ**, Kodama T, du Lac S. (2017) Molecularly distinct types of projection neurons in the medial cerebellar nucleus mediate diverse outputs of the cerebellar vermis. The 40th Annual Meeting of the Japan Neuroscience Society, Makuhari, Japan. Jul 20-23, 2017.

Fujita H, **Sima RJ**, Kodama T, du Lac S. (2017) Molecularly and anatomically distinct types of projection neurons in the medial cerebellar nucleus mediate diverse outputs of the cerebellar vermis. 8th International Symposium, Society for Research on the Cerebellum and Ataxia, Winnipeg, Canada. May 24-26, 2017.

Sima RJ, Fujita H, du Lac S (2016) Medial cerebellar nucleus projects to mesopontine cholinergic neurons. Johns Hopkins University Neuroscience Retreat. [Poster]

Sima RJ, Nadal-Vicens MF, Kravitz EA (2012) A novel model of social defeat: the divergent effects of lithium and valproic acid in *Drosophila melanogaster. Harvard Mind, Brain, Behavior Workshop Seminar*. [Talk]

Sima RJ, Nadal-Vicens MF, Kravitz EA (2012) A novel model of social defeat: the divergent effects of lithium and valproic acid in *Drosophila melanogaster. Harvard Prefrosh Science Symposium*. [Poster]

Sima RJ, Nadal-Vicens MF, Kravitz EA (2011) A *Drosophila* social defeat model for depression. *Harvard College Program for Research in Science and Engineering (PRISE) Fellow Presentation Series.* [Talk]

Sima RJ, Nadal-Vicens MF, Kravitz EA (2010) A *Drosophila* behavioral paradigm as a model to test the effects of psychotropic modulation on social defeat. *Annual Harvard Undergraduate Research Symposium*. [Poster]

Sima RJ, Nadal-Vicens MF, Kravitz EA (2010) Using a *Drosophila* behavioral paradigm as a model to test candidate psychiatric disease genes. *Harvard Prefrosh Science Symposium.* [Poster]

Sima RJ, Nadal-Vicens MF, Kravitz EA (2009) Using a *Drosophila* behavioral paradigm as a model to test candidate psychiatric disease genes. *Annual Harvard Undergraduate Research Symposium*. [Poster]

Sima RJ (2009) In Praise of Rose-Tinted Glasses: The Necessity of Self-Deception. *12th Annual National Undergraduate Bioethics Conference.* [Invited Talk]

Teaching Experience

Instructor – Science Writing workshop, Johns Hopkins University (JHU) **Fall 2018**
Teaching Assistant – Neuroscience of Pain, JHU undergraduate course **Fall 2015**
Teaching Assistant – Neuroscience and Cognition II, JHU graduate course **Spring 2015**
Teaching Assistant – Human Neuroanatomy, JHU Medical Program **Spring 2014**
Seminar Leader – Harvard Summit for Young Leaders in China Conference **Summer 2012**
Assistant Instructor – Cold Spring Harbor Laboratory Course in Neurobiology of Drosophila **Summer 2011**
Invited Guest Lecturer - Harvard Foundation's Annual Albert Einstein Science Conference **Spring 2010**

Leadership and Outreach Experience

President and Co-Founder, Johns Hopkins Science Policy Group **2016 – 2018**
Contributing Writer, Johns Hopkins Medicine Biomedical Odyssey Blog **2015 – 2016**
Communications Chair, Project Bridge **2013 – 2017**
Co-Chair (2011-2012), Harvard Society for Mind, Brain, and Behavior **2008 – 2012**

Web Managing Editor (2010-2011), The Harvard Brain magazine **2009 – 2011**
Co-Director (2010-2011), Harvard ExperiMentors **2008 – 2012**

Affiliations

Society for Neuroscience, Member **2014 – present**
American Association for the Advancement of Science, Member **2016 – present**
American Society for Biochemistry and Molecular Biology, Member **2017 – present**
National Science Writers Association, Member **2018 – present**
American Physiological Society, Member **2018 – present**